ENDOCRINOLOGY

THE BULLETIN OF THE ASSOCIATION FOR THE STUDY OF

INTERNAL SECRETIONS

Volume 19, Number 3 MAY-JUNE, 1935 Serial No. 101

MENSTRUAL DYSFUNCTIONS IN DISORDERS OF THE PERSONALITY: THEIR NATURE AND TREATMENT*

EDWARD B. ALLEN, M.D.
Bloomingdale Hospital
WHITE PLAINS, N. Y.

This paper presents a clinical study of the relation of menstrual disorders to functional mental illnesses and the effects of treatment, especially endocrine, for their relief. The cases of 150 patients at Bloomingdale Hospital have been selected. The selection was dependent upon whether or not the medical and laboratory examinations gave adequate data to be of value. No case record was selected that covered a period of less than four months. As far as possible in this study all factors, which might contribute to menstrual disturbances, but which at the same time might cloud the issue, were taken into consideration. Cases having complicated physical conditions obviously influencing the personality deviation and possibly also the menstrual cycle were eliminated. Complete physical examinations with many laboratory tests performed by the resident physicians were checked in detail by the attending consultants, including the internist and gynecologist. Blood Wassermann findings in all cases were negative. Blood sugar and urea determinations were made in 81 per cent of the cases and were within the usual average limits. Blood calcium tests were performed in only 10 per cent of the cases but no abnormal findings were obtained. Basal metabolic tests on approximately 55 per cent of the group ranged from minus 27 per cent to plus 15 per cent. The variations in the last findings had a closer correlation to the emotional state of the patient than to any objective physiological condition.

The 150 cases have been divided into four groups, according to psychiatric diagnosis, as follows: schizophrenic psychoses, 54 cases; manic-depressive psychoses, 54 cases; psycho-neuroses, 21 cases, and miscellaneous, 21 cases. The last group was mainly composed of pychopathic personalities, with or without psychoses, and two cases of psychoses associated with somatic disease. The psychopathic personalities were patients

*Read before the Eighteenth Annual Meeting of the Association for the Study of Internal Secretions, Cleveland, Ohio, June 11, 1934.

who had been hospitalized beause of minor asocial tendencies such as lying or stealing, because of depressive, excited, or paranoid episodes, or because they had sought, through alcohol or drugs, an escape from psychological conflicts. The psychoses associated with somatic disease were in patients who, when the somatic factors were removed, showed depressive or residual schizophrenic traits.

The ages of the patients studied varied from 14 to 50 years. The average age of onset of menstruation in each group was essentially the same: 13 years for the first three groups and 14 years for the miscellaneous one.

The following general observations were outstanding in studying these groups. No difference was observed in the character or duration of the menstrual flow prior to the onset of mental illness in those patients who menstruated regularly with a moderate flow during their hospital residence. It was in the schizophrenic group that menstrual irregularities prior to the onset of the illness were most marked. An occasional patient, with a history of amenorrhea on admission, did not menstruate for a few months until the normal weight was regained, but the gain or loss of weight was seldom enough to influence the menstrual function. Sleep also appeared to have no direct relation to menstruation.

Patients often entered the hospital in acute states of excitement. It was interesting to note that many of them were menstruating at the time. The significance of this will be clear later. Of the four groups 76 per cent of the patients in the second group, the manic-depressive, were either menstruating on admission or did so within a week. Here, of course, the emotional factor was easily recognized. Only 46 per cent of the schizophrenic group were menstruating either on admission or did so within a week, while among the psychoneurotic and miscellaneous groups the percentage was even less, 43 per cent.

The question arose as to whether further study would reveal any relation between the emotional factor present in a mental illness and the character of the menstrual function. In a previous study (Allen and Henry) it has been shown that menstrual dysfunctions do present somewhat different characteristics in the various types of personality reactions.

Let us consider first the complex and varied schizophrenic group. If the psychotic reaction was acute, we found marked irregularities in the duration and amount of the flow, and in the intervals between the periods. Amenorrhea was most likely when the patient was confused, depressed, desperately suicidal, perplexed or stuporous. It was generally associated with a reduction of muscular activity and with physical depletion. Profuse menstruation, on the other hand, was more likely to occur when the patient was continuously active and better nourished. In the sub-acute or chronic cases, the irregularities were less marked and menstruation usually was fairly regular, when all acute symptomatology had subsided.

Physical constitutional inadequacies were outstanding in this group. A masculine hair distribution was present in 35 per cent, and the gynecologist found indications of underdevelopment of the sexual organs in 31 per cent.

Among the manic-depressive psychoses, our second group, there was a definite correlation between the degree of activity, with its associated mood, and the amount of the menstrual flow. Of 34 patients observed in the manic phase, few showed any interruption in their menstrual periods. In several of the hypomanic patients the flow was more profuse and of longer duration than usual, but it did not interfere with their activities. The more intensely excited manics occasionally skipped a period.

The most characteristic reaction in the 43 cases observed in the depressive phase was amenorrhea, which was directly associated with the duration of this phase and with the intensity of the mood. As the depression became more pronounced the intervals between the periods became longer, the flow became scant and of shorter duration, and finally a period of amenorrhea intervened. The degree of psychomotor retardation appeared to be the essential index of whether the menstrual periods were to be delayed or absent. In the group studied the retardation appeared to be more pronounced in the young and unmarried patients, in whom amenorrhea was often present. In married women with depression but little retardation, cessation of the menstrual flow was rare, particularly if they had borne children.

Of the manic-depressive patients 24 were observed during both phases of the illness (i.e., exaltation and acute depression) and many of them through several attacks. The menstrual reactions in these patients were characteristic of the mood phase of the illness as outlined above. In other words, when elated or overactive there was little interference with menstruation; when depressed and retarded, amenorrhea developed.

In the third group, the psychoneurotics, dysmenorrhea was a most distressing menstrual symptom. The patients requiring hospital residence are usually more severely compromised than the average psychoneurotic living outside of the hospital. Those who were markedly depressed and displayed suicidal tendencies generally had amenorrhea over periods ranging from one month to a year, similar to the reaction already noted in the acute depressions. When menstrual irregularities occurred a tendency to schizophrenic traits was evident in the personality deviation.

In the miscellaneous, and last, group many of the psychopathic personalities had only slight menstrual dysfunctions, but all experienced some discomfort. The interval between periods was often irregular. Periods were often prolonged to six or seven days. Sometimes the flow was profuse and dysmenorrhea was usually present, as in the psychoneurotic group.

Generally speaking, in the four groups there seemed to be no consistent sequence in the appearance or disappearance of painful emotional

states and the menstrual disorders. These phenomena seemed to be concurrent in most of the patients, and it was not possible to say definitely that either one preceded or followed the other. As a rule, however, minor emotional changes can be observed for a considerable period of time before menstrual functions appear to be disturbed. On the other hand, the return of menstruation is a favorable prognostic sign that a readjustment, both physically and psychologically, is taking place.

The nature and the treatment of menstrual dysfunctions in personality disorders are so closely inter-related that it is impossible to discuss treatment adequately without bearing in mind the various aspects of the problem as briefly sketched above. Treatment is obviously dependent upon the way in which the dysfunctions manifest themselves, upon the course they run, and upon close observation of the factors which appear to facilitate a return to a normal rhythmic function. The psychological and the physiological levels of the disintegration must be studied and treatment must be directed toward a normal restitution of both of these disturbed levels. For the purposes of this study we stress the physiological aspects in our discussion, although the influence of emotional factors upon the general behavior pattern of the patient needs serious attention in the total therapy.

In considering menstruation from the endocrine point of view our interest should be directed to the sexual cycle. We should think of menstruation in relation to the other changes taking place in the sexual cycle instead of treating it as a more or less isolated phenomenon. It is our one clinically-objective manifestation of the sexual cycle, but it contributes little to our understanding of the associated morpho-physiological changes of the ovary and the endometrium. It is only recently that attempts have been made to fully understand these changes and to perfect clinical methods of investigation for determining the same.

With the sexual cycle of the female dependent upon two hormones or activating substances from the anterior pituitary, and two from the ovary, and with the necessity of the added hormonic activity from the posterior pituitary in the advent of pregnancy, it can be readily seen that the question of relief for any dysfunction of this cycle through endocrine therapy is complicated, irrespective of whether the symptoms are chiefly emotional or physiological in character. The reported results in the handling of definite physical symptoms are conflicting. In personality disorders we deal with intangible and ever-varying emotional and instinctive disturbances. It is extremely difficult to know what effects relief, or what is at fault when relief is not obtained. In the 150 cases included in this study, 56 patients received endocrine therapy in addition to some form of psychotherapy. The other 92 subjects were treated without endocrine therapy and serve as a control group. The fact that only a little over one-third of our patients presented symptomatology, which seemed to warrant endocrine therapy, bespeaks a conservative attitude.

Only in rare instances were the patients in either the control group or in the group treated with endocrine therapy given any drug medication, and then only for a short period.

The type of mental reaction and the psychological attitude of the patient had a very definite bearing upon the amount of endocrine therapy they received. Only 15 of the 54 schizophrenics received glandular therapy. Many were too disturbed. When they became less so the menstrual function had become more regular so that such treatment was not indicated. Patients would often accept such therapy at one time and refuse it at another, thus interrupting the course of the treatment.

In reviewing the case records of these 15 patients it was found that those who were acutely ill received treatment for comparatively short periods, or that treatment had been given with first one glandular product and then another. The variable menstrual reactions in the disturbed schizophrenics made it difficult to treat the patient for any one particular menstrual dysfunction for any great length of time. There were four patients, however, in this group whose reactions were sufficiently subacute that their accompanying menstrual dysfunctions were not subject to the variations just noted. Endocrine therapy could be instituted with some definite objective in mind, and each patient presented a different therapeutic problem worthy of note.

Case 1. A stout woman of 47, with a marked paranoid trend, gave a history of maturing at 14. Her menses were regular until the onset of the menopause at 44, when her periods became irregular. They ceased altogether three months before admission, after three weeks of scanty, irregular bleeding. She was given three injections of amniotin, 1 cc. intramuscularly every other day, on three different occasions about a month apart—a total of 90 Allen-Doisy units. Some time after each series of injections she menstruated but the periods were scant. They ceased when the injections were stopped.

The results in this case suggested that a woman can be forced to bleed in the menopause, especially if the menses have stopped only a short time before, but there is no good reason why the menopause should be prolonged. There was a slight improvement in the patient's psychotic condition so that she was eventually able to live outside of the hospital under supervision, but there was no indication that this had any relation to the forcing of uterine bleeding, probably menstrual in character.

Case 2. A single woman of 36, with infantile genitalia, who gave a history of having menstruated only four times in her life, and who was in a depleted physical state, was given ovarian extract gr. V. twice daily for a month, theelin (1 cc.) intramuscularly for six days, alternating injections of follutein (total of 1850 Zondek-Aschheim units) and amniotin (total of 266 units) over a period of three months, and progynon tablets (one t.i.d.) for two months, without re-establishment of the menstrual flow.

This case illustrates the uselessness of endocrine therapy in a case where permanent arrest in genital development has occurred. The response might have been more favorable if the woman had been treated in early adolescence.

Case 3. A girl of 17 was admitted in a catatonic stupor. She had already been ill for seven weeks and had not menstruated for a month. Two months after admission she was given ovarian extract gr. V., corpus luteum gr. II, and thyroid extract gr. II, three times daily for five months. She also received anterior pituitary gr. I, three times daily for about two months. Five months later, when she came out of the stupor, her menstruation was re-established. All glandular therapy was discontinued. She skipped the next period and had irregular painful menstruation until she left the hospital nine months later in a complete remission. She returned one and a half years later in another catatonic stupor with a history of suppression of the menses for five months.

No glandular therapy was given at this time, but five months later she came out of the stupor and had a rather scant menstrual flow for four days. She skipped the next period but her menses then became regular.

Had we not followed this case beyond the first hospital residence we would have been inclined to believe that the endocrine therapy had been helpful in re-establishing the menstrual flow. Perhaps it was, but in her second attack the menstrual irregularity corrected itself without endocrine therapy. Prolonged observation of our patients, not only when under endocrine therapy but subsequently, is therefore necessary.

Case 4. A girl of 16 entered the hospital with a history of irregular menstruation since its onset at the age of 14. She was given ovarian extract gr. V, corpus luteum gr. II, once daily for five months, when leukorrhea was reported for one day. The dosage of ovarian extract and corpus luteum was then doubled for 16 days. Then progynon tablets—one twice daily—were given for 43 days, when they were discontinued as the patient had a menstrual period lasting two days. No further endocrine therapy was given. After an interval of 44 days there was another period lasting three days. The patient left the hospital soon after. She had gained nine pounds in weight, and had improved physically during her hospital stay. She was active, athletic, and eating well when she left.

As many of our patients have had a restoration of menstruation with improvement in their physical condition it is doubtful whether the endocrine therapy was helpful in this case.

In the manic-depressive group 19 of the 54 patients studied received glandular therapy. Here the endocrine treatment was instituted in the majority of the cases to bring about a restoration of menstruation that had been suppressed with a depressive mood. In no instance, no matter how many ovarian preparations were given, even when augmented by pituitary therapy, did they directly hasten the return of menstruation, or shorten the duration of the depression. Of course, in all of these cases other supportive measures were tried. With the lifting of the depressive mood, menstruation was re-established.

In the psychoneurotic group all except four (21 in all) received various forms of glandular medication. These patients requested treatment. Many had received glandular therapy before coming to the hospital and told others about it. Much of the glandular medication given in this group was not so much to effect a menstrual change as to relieve subjective discomfort. Thyroid was given in many of these cases. It raised the pulse rate if given in sufficiently large doses but had little effect upon the basal metabolic rate when it approached normal limits. It was discontinued when some new form of glandular therapy was tried or the patient had sufficient insight to realize that her difficulties were largely psychogenic.

Of the patients in the miscellaneous group only a third (seven in number) received glandular medication, chiefly for dysmenorrhea, with indifferent success. One patient, a psychopathic personality without psychosis, was given alternating injections of follutein (anterior pituitary sex hormone) and saline solution without her knowledge. On two occasions after the saline injections she complained of more pain, but her statements varied and she often had pain after the injections of follutein. At any rate, she was willing to stop the treatment and to submit to a modified psychoanalysis soon afterward. Another patient, a girl of 22, received ovarian extract gr. V. t.i.d. 14 months. Her periods continued regular during this time but the dysmenorrhea was not relieved.

The miscellaneous group of patients was generally active, out-of-doors, and in the main had relatively little menstrual disturbance. Most of them came to the hospital for relief of personality problems that were not associated with marked subjective discomfort.

The general indications for endocrine therapy have been for the restoration of menstruation in prolonged cases of amenorrhea, and for the relief of dysmenorrhea, menorrhagia, and metrorrhagia.

In cases of amenorrhea, irrespective of the type of personality disorder, treatment directed toward improving the general health of the patient has produced the best results. We seek to correct both somatic and behavioristic disorders. Special attention is given to the improvement of nutrition, to proper fluid intake, satisfactory elimination, sufficient rest and quiet during stages of excitement, to a carefully-graded and increased activity for the depressed and retarded, and to an awakening of interest in the apathetic. Possibly larger doses of amniotin or some other form of ovarian sex hormone which can be given in concentrated form may be of use where there is uterine hypoplasia. We have not had experience with any such cases as yet. The fact that menstruation returns with the restoration of a more comfortable mood, even after a prolonged amenorrhea, speaks against hypoplasia in the cases we have studied.

Dysmenorrhea has been extremely variable in personality disorders. The patient's mental attitude has appeared to be an important problem. This attitude has been influenced by early environment and training. A change of attitude and the lessening of any accompanying emotional stress, with direction of the patient's attention to some external interest, has been more beneficial than endocrine therapy.

The problems of amenorrhagia and metrorrhagia have given us little difficulty in the past. We have promptly confined these patients to bed and the flowing, if there was no organic disease of the ovaries or uterus, has generally stopped in six or eight days. But of late we have found that menorrhagia was becoming a problem in convalescent patients who desired more activity. In these functional cases we have been trying antuitrin-S, with the supposition that the profuse and prolonged flow is due to a persistent graafian follicle that does not luteinize, or to a defective or functionally inactive corpus luteum. The objective is to convert the persistent graafian follicle into a corpus luteum and evoke in the latter the production of progestin to neutralize the untoward effects of the ovarian hormone, oestrin, upon the endometrium.

At first we met with little success but it would appear that we were too easily discouraged and did not push the therapy sufficiently. After a few injections the patients would have another profuse period, and we would question whether the therapy had not augmented the flow. We have recently started treating these functional cases more intensively by daily injections of 1 cc. (100 rat units) of antuitrin-S. The injections are given continuously for periods of two to three months, including the

menstrual periods. To overcome the patient's objection to daily subcutaneous injections, which are apt to be painful, we have been giving them with larger needles in the buttocks intramuscularly. Some patients have spoken of feeling better, and of the flow being less, but our charts thus far have shown little, if any, lessening of the duration of the periods.

We are now confining our glandular therapy for the relief of menstrual dysfunctions to amniotin and antuitrin-S, not because we doubt the efficacy of the other products if given in sufficient dosage, but because we can give these products in greater concentration in smaller doses. This is less troublesome to the patient and lessens the expense.

It would be practically impossible to treat any of our patients with endocrine therapy alone. As a result the relative value of glandular therapy and other forms of therapy cannot be determined with precision. In all forms of treatment of the mentally sick the power of suggestion is very important. In addition we modify all forms of therapy to meet the problems of the individual case. We try to remove emotional resistances to menstruation and thus contribute to subjective relief. Psychotherapy includes participation in varied activities with other individuals in order to effect a socialized adjustment.

Limited space does not permit the presentation of a detailed correlation of menstrual phenomena with data obtained from the clinical records in a large number of cases. Individual variations in the menstrual charts tend to confuse the casual observer, but a careful study of the clinical record, along with the menstrual change, makes these variations more intelligible.

Four menstrual charts have been selected for presentation, one from each of the specified groups, which may be regarded as fairly typical of that group. The spaces between the vertical lines show the days of the month and the duration of the individual menstrual periods. Each horizontal division represents the passage of two months. Whenever the original chart specified the amount of the flow this has been indicated by the width of the areas in black. If the flow were profuse, the whole space indicating a month was made black, while if the flow were scanty only one-third of the space was made black. A normal or unspecified amount of menstrual flow has been indicated by making two-thirds of the space black.

Accompanying each chart are some notations from the clinical records, so that delay, irregularity, or absence of menstruation and the effect of endocrine therapy upon the same may be correlated with the patient's condition.

Case C. M. J. was a single girl 21 years of age. She was diagnosed as suffering from schizophrenia of the catatonic type. The menses began at 15 and had been regular. She was admitted to the hospital in October, 1930. She was restless and hearing voices but was amenable and pleasant. The menses were regular. In November she was apprehensive, depressed and tense. She did not menstruate during the next six months, was negativistic and had to be tube-fed during this period. In June, 1931, she was gradually improving and was playing tennis. The menses returned, but for the next six months the periods were irregular. The patient was fairly active. In January, 1932, she was mildly

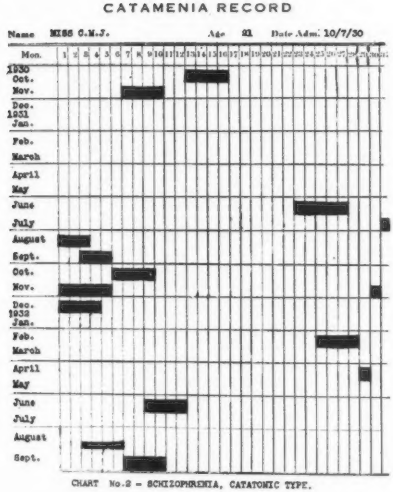

CHART No.2 - SCHIZOPHRENIA, CATATONIC TYPE.

depressed and self-accusative. She did not menstruate. In February there were short periods of exhilaration alternating with despondency and in March she was stuporous and there were no menses. In April she was cataleptic, her hands were cold and cyanotic and she was incontinent and mute. In June she was showing more initiative, and in August was more alert. In September she was overactive and impulsive and the menstrual period was profuse. This patient received corpus luteum gr. II once daily and ovarian extract gr. V t.i.d. from February 5 to June 28, 1931, during her first period of amenorrhea. No further glandular medication was given. She left the hospital July, 1933, socially adjusted, and with regular menstrual periods.

Case H. R. was a manic-depressive patient of the cyclic type. She was 19 years old and single. The menses began at 13, and occurred at intervals of a little under four weeks. She was admitted February, 1932, in a hypomanic mood, but with a history of previous depressive mood phase, when she had ceased to menstruate. Menstruation had been re-established with the onset of the hypo-

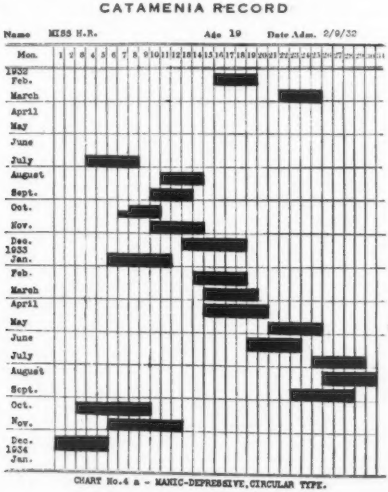

CHART No.4 a - MANIC-DEPRESSIVE, CIRCULAR TYPE.

manic mood in December, 1931. At the time of admission she was talkative, overactive and euphoric. In April she was depressed, slightly retarded and did not menstruate for the next three months. She was overactive and talkative in July and the menses were re-established. In January, 1933, she was slightly depressed and remained so until May, but was able to keep up her routine activities and be on a convalescent hall. The menses were somewhat irregular, but the flow was not diminished. From May to November she was hyperactive, talkative and was in a euphoric mood. In November she became depressed and tired easily. She was still depressed in January and was retarded and self-depreciative. She did not menstruate. This patient received antuitrin-S—10 doses of 1 cc. daily following menstrual periods in October, November and December, 1933, for prolonged menstruation and dysmenorrhea with little effect. The menses ceased with subsequent depression.

Case R. E. was a single woman, aged 41, suffering from psychoneurosis of the psychasthenic type. The menses began at 12 and had been regular. She was admitted to the hospital in May, 1932. At that time she was agitated and tense. In June she felt more comfortable but still had many fears. She was quieter in July but in August the old ideas were returning and she felt hopeless. There was a two-day menses at this time. In September she reacted well to interview. In November she wakened early and had a choking feeling and disturbing

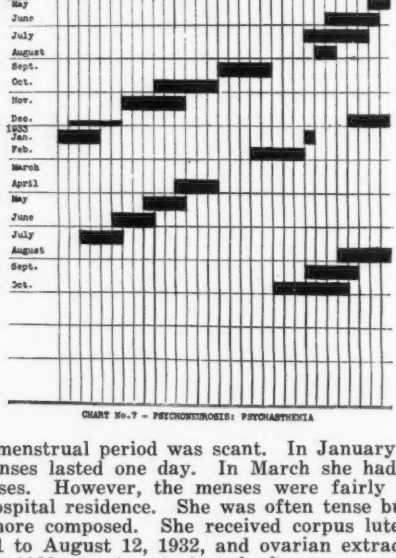

CHART No. 7 – PSYCHONEUROSIS: PSYCHASTHENIA

dreams. The next menstrual period was scant. In January she had disturbing dreams and the menses lasted one day. In March she had erotic dreams and there were no menses. However, the menses were fairly regular during the remainder of her hospital residence. She was often tense but less agitated and gradually became more composed. She received corpus luteum gr. V. t.i.d. by mouth from July 11 to August 12, 1932, and ovarian extract gr. V. t.i.d. from April 24 to June 14, 1933—treatment started after one normal period following amenorrhea.

Case G. A. was a woman with a psychopathic personality. She was 35 years old and married. Her mental disorder was at first thought to be hysteria but symptoms of this were only episodic. The menses began at 12 and had been regular (4-7 days). She was admitted to the hospital in December, 1933, complaining of fatigue, insomnia and diarrhoea. In January, 1934, she was less tired, performed more active routine and was comfortable during the menses. In February she became irritable with a depressive trend. She had diarrhoea and the menses were profuse. She was less uncomfortable during the menstrual period in March but felt worse in April and was irritable and depressed. She was still depressed in May and was tense, tearful, and did not follow routine during the menses. She had menstrual discomfort at the onset, but not as severe as in previous period. She received continuous daily injections, intra-

muscularly, of antuitrin-S, from February 16 to May 24, 1934. According to the patient's statement she has been helped. She states that the flow has been less each day but that the duration of the periods has not been lessened. She has not had to rest in bed during each of them. This case indicates the necessity of checking up the subjective impressions of the patient with as objective manifestations of the same as it is possible to determine.

CATAMENIA RECORD

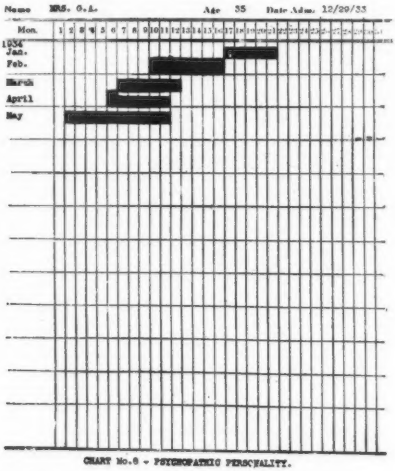

CHART No. 6 — PSYCHOPATHIC PERSONALITY.

CONCLUSIONS AND DISCUSSION

In this clinical study of the relation of menstrual dysfunctions to personality disorders, all factors which might contribute to menstrual disturbances were taken into consideration as far as it was possible to do so. Those cases disclosing a physical condition or illness complicating the mental picture were eliminated. Of the factors remaining for consideration, the emotional status was the only one that appeared to have a definite correlation, both quantitatively and qualitatively, with variations in the menstrual function. These menstrual variations seemed to be somewhat characteristic of the different types of personality disorders, but only because of the different emotional reactions which they presented.

The emotional stress was variable and unpredictable in the acutely sick schizophrenic patients, and was accompanied by similar unpredictable variation in the menstrual function. In the sub-acute or chronic schizophrenics, the emotional factors were less marked and varied, as also were the menstrual dysfunctions. This variability made endocrine therapy difficult and tended to obscure any value it might possess.

In the manic-depressive patients, the hypomanic mood was accompanied by much activity and often with profuse and sometimes prolonged menstrual periods. The disturbed manics were subject to more distressing stimuli. They would occasionally skip a menstrual period. As cessation of the profuse periods resulted from lessening their activity, glandular

therapy was not indicated. The patients in the depressive phase of this psychosis showed a variation coördinate with the depth and duration of their depression and the diminution and suppression of their menses. In no case did endocrine therapy directly shorten the period of amenorrhea or increase a diminished flow.

The psychoneurotics, who are subject to rapid variations in mood, usually of much less intensity than the psychotics, were most apt to be troubled with dysmenorrhea, but excessively painful emotional stimuli often led to a suppression of menstruation as in the case of the schizophrenics or manic-depressive patients. It is difficult to get the psychoneurotics' assistance for any form of glandular therapy for prolonged periods, but where obtained results were not gratifying. More satisfactory results have been achieved by changing their mental attitude and directing their attention to some external interest.

The miscellaneous group was made up largely of psychopathic personalities. They sought relief from their emotional discomforts more often in asocial behavior, alcohol or drugs, than in a symptomatology accompanied by evidences of disturbed physiological functions. Emotional stress in these cases was frequently accompanied by dysmenorrhea or slightly prolonged and occasionally somewhat profuse periods. We have used antuitrin-S over prolonged periods with subjective relief and some diminution of the profuse flow in these patients, but have not shortened the duratioin of their periods.

In personality disorders the problems are much more complicated than those arising in the treatment of menstrual dysfunctions in general medical, or in gynecological practice. There the attention is focused on the physiological disturbance and treatment is directed toward a reintegration of the disordered physical factors. In personality disorders the attention is directed, not only toward these factors, but to what is wrong with the thought processes, and to how this is modified by feeling. Menstruation should be a physiological activity unaccompanied by pain or discomfort other than the attention it necessitates to the hygienic control of the same.

Menstrual dysfunction is only one of many physiological ways in which the endocrine system expresses emotional disturbance in a disordered personality. A most important consideration is the reduction of emotional stress. If this is done the menstrual dysfunction will be corrected without specific drug therapy, unless there is some underlying endocrine or structural disease. Such disease may be present. Our experience at Bloomingdale Hospital leads us to believe it is exceedingly rare in functional personality disorders.

We are apt to pay so much attention to such an objective phenomenon as menstruation that we overlook other factors. It is just as rational to think of relieving a patient of a psychosis or neurosis by a high enema to effect a bowel movement, as to expect relief by forcing menstruation. It

is even doubtful whether a physically-depleted woman, or one with underdeveloped genital organs, should be forced to bleed from her uterus. With the limited knowledge we now possess regarding the inter-relationship of physiologic disorders and abnormal mental conditions we are unable to say to what extent amenorrhea may be a protective mechanism. One of the hopes for the endocrine treatment in the future is that it will stimulate further genital development in those cases in which the whole sexual organization is still immature, and that attention will be directed to the correction of menstrual irregularities at adolescence, the history of which is so often obtained in the adult with menstrual dysfunctions. Conservatism is still necessary. It would be unfortunate if progress were delayed by too hasty conclusions regarding therapeutic results.

SUMMARY

1. In a study of the cases of 150 patients at Bloomingdale Hospital, suffering from personality disorders, the emotional factor was the only one that appeared to have a definite correlation, both quantitatively and qualitatively, with variations in the menstrual function. The fact that these menstrual variations seemed somewhat characteristic of the different types of personality disorders was due to the different emotional reactions which they presented.

2. A depressive mood, physical depletion, and motor underactivity are conducive to amenorrhea, irrespective of whether or not the patient is delusional or hallucinating. An expansive, elated, or ecstatic mood, with good physical condition and motor overactivity, predispose to a profuse and prolonged menstrual period. Agitation and worry are more apt to be associated with a profuse than a decreased flow. These various conditions are often intermingled and thus complicate the type of menstrual dysfunction and cause it to vary from time to time.

3. Treatment directed toward promoting the general health and alleviating the emotional distress of the patient was productive of the best results in menstrual dysfunctions associated with disorders of the personality. In no case did endocrine therapy directly shorten the period of amenorrhea or increase a diminished menstrual flow. In cases of dysmenorrhea and of profuse or prolonged menstruation, antuitrin-S offered subjective relief and appeared to diminish the flow, but did not shorten the duration of the period.

REFERENCES

Allen, E.: Sex and the Internal Secretions. Williams & Wilkins Co., Baltimore, 1932.

Allen, E. B. and G. W. Henry: The Relation of menstruation to personality disorders. Am. J. Psychiat. **13**: 239. 1933.

Brush, A. L.: Menstruation in Women: Average Control. To be published.

Burch, J. C., W. L. Williams, J. M. Wolfe and R. S. Cunningham: The hypophyseal-ovarian relationship; quantitative studies with especial reference to hyperplasia of endometrium. J. A. M. A. **97**: 1859. 1931.

Chadwick, M.: The psychological effects of menstruation: Nerv. and Ment. Dis. Mon. No. 56: New York and Washington, 1932.

Corner, G. W.: The nature of the menstrual cycle. Medicine **13**: 61. 1933.

Englebach, W.: Endocrine Medicine. Charles C. Thomas, Springfield, Illinois, and Baltimore, 1932.

Ewart, C. T.: Amenorrheal insanity. J. Ment. Sc. **58**: 76. 1912.

Frank, R. G.: The Female Sex Hormone. Charles C. Thomas, Springfield, Illinois, 1929.

Fulkerson, L. L.: The endocrine glands in relation to Gynecology. New York State M. J. **31**: 873. 1931.

Graves, W. P.: Female Sex Hormology. W. B. Saunders Co., Philadelphia and London, 1931.

Horney, K.: Research in Female Psychology. Institute for Psychoanalysis. Chicago, Illinois. (Review 1922-33). 1931.

Hoskins, R. G. and F. H. Sleeper: The thyroid factor in dementia praecox. Am. J Psychiat. **10**: 41. 1930.

Hoskins, R. G.: Some principles of endocrinology applicable to organotherapy. J. A M. A. **79**: 104. 1922.

Mackenzie, T. C.: Menstruation in the insane. J. Ment. Sc. **54**: 116. 1908.

Marañon, G.: The Climacteric. C. V. Mosby, St. Louis, 1929.

Mayer, C. and L. Goldstein: Clinical Endocrinology of the Female. W. B. Saunders Co., Philadelphia and London, 1929.

Novak, E.: The bearing of recent work on the anterior pituitary hormone upon gynecological problems with especial reference to disorders of menstruation. Endocrinology, **15**:. 273. 1931.

Pratt, J. P.: Ovarian therapy. Endocrinology, **16**: 45. 1932.

Schoeller, W., M. Dohrn and W. Hohlweg: The possible clinical indications for follicular hormone therapy, based upon its known biologic effect in animal experiments. Am. J. M. Sc. **182**: 326. 1931.

Seward, G. H.: The female sex rhythm. Psychol. Bull. **31**: 153. 1934.

Smith, G. Van S. and O. W. Smith: The role of Progestin in the female reproductive cycle. J. A. M. A. **97**: 1857. 1931.

Strachan, G. I. and I. Skottowe: Menstruation and the menopause in mental disease. Lancet, **1**: 1058. 1933.

CHANGES IN THE ENDOMETRIUM AFTER FEMALE SEX ENDOCRINE THERAPY: A CONSIDERATION OF THE EFFECT OF ANTUITRIN-S IN HUMAN SUBJECTS*

JOHN ROCK, M.D.
From the Free Hospital for Women
BROOKLINE, MASS.

I wish to present a few clinical facts which suggest the soundness of Novak's theory that there is a bleeding factor in humans, and which also suggest that at least the immediate relief of dysfunctional uterine bleeding by pregnancy urine extract or P. U. is not attributable to any luteinizing effect on the ovarian follicles.

Because of the expense and the inconvenience of extracting hormones from blood and urine, and because of the inaccuracy of the biological tests of these extracts as exact indicators of ovarian activity we have sought more nearly to estimate the degree of follicular activity by study of the endometrium. Here we have a tissue the cytological morphology of which, as far as we know, is entirely under the control of the specific hormones from the follicle and the corpus luteum. We have used a modification of the Klingler-Burch suction curette to obtain fragments of the endometrium from various parts of the inner uterine wall.

Studies of such endometrial specimens incident to our attempts to produce artificial menstruation in patients with long standing amenorrhoea have convinced us of the dependability of this method. Werner and Collier, and Kaufmann have shown that estrin causes proliferation in the human endometrium as it does in that of monkeys and lower animals, and Kaufmann has shown that progestin, following estrin, brings forth the anticipated secretory changes in this proliferated endometrium. We have repeated Kaufmann's work with young anatomically intact patients with amenorrhoea, however, instead of with castrated women, or women past the menopause.

Three such cases follow.§ The first patient, L—108/67, was 33 years old and had one child in 1925. Her catamenia came every three or four months until amenorrhoea began, three years before treatment. At this time the suction specimen was characteristic of the specimens obtained from a long quiescent endometrium as only superficial epithelium and practically no stroma was obtained. Following this the patient was given 10,000 r.u. of Progynon-B† on five alternate days. Then, beginning five days after the last dose, she was given, on four successive days, 10 rabbit

*Read before the Eighteenth Annual Meeting of the Association for the study of Internal Secretions, Cleveland, Ohio, June 11, 1934.

§These, with other cases, and with photomicrography of the endometria studied have been already reported in the New England Journal of Medicine, **210:** 1303-1310. 1934.

†We are indebted to Schering Corp. of New York for a generous supply of this hormone and of Proluton.

units of Proluton, Schering's preparation of progestin. On the last day another specimen of endometrium was taken. The glands were now hyperplastic, with swollen vacuolated tall columnar epithelial cells. The stroma was dense but there was swelling of many stroma cells, and widespread extravasation of blood. This was an early secretory endometrium. Two days after the last injection the patient had a normal but scant menstruation.

The second patient, R—109/122, was 29 years of age, had one child in 1929 and amenorrhoea since then. Before treatment in 1934, her endometrium was atrophic. From Jan. 12 to Jan. 26 she was given five doses each of 10,000 r.u. of Progynon-B. On each of five successive days beginning Jan. 30 she was given 10 rabbit units of Proluton. On the day of the fourth injection the endometrium already clearly showed the effect of progestin, in dilated serrated glands with vacuolated epithelium containing large round nuclei. On Feb. 4, the day after the last injection, she used seven napkins, the beginning of a three-day menstruation.

The following experiment shows the effect first of estrin alone and then the changes in the proliferative endometrium after such a small amount of progestin as forty units.

The third patient, C—573, was 26 years old, barren and complaining of amenorrhoea since September, 1932. Before treatment in March, 1933, her endometrium was of the characteristic thin, almost atrophied, type. After five doses of Progynon-B, each of 10,000 r.u., given over a period of two weeks, it had developed into an early proliferating endometrium with small regular glands lined by compact columnar epithelium and with dense stroma of small cells. Even from this dosage the patient used two napkins a day for five days, the flow beginning two days after the last injection. Two months before this treatment a similar one had been given, but after the Progynon, five doses each of 10 rabbit units of Proluton were given. A specimen obtained after the Proluton showed the epithelial cells changing from columnar to cuboidal and the glands dilating. The stroma was less dense and the nuclei were swollen. This is characteristic of a very early progestin change. It was followed in two days by a five-day period in which ten napkins were used.

If, then, even a small amount of luteinization occurs in the follicles of the patient with dysfunctional flowing after urinary prolan is given, we might expect an amount of progestin thus formed, sufficient to inhibit the flow, to show a characteristic change in the endometrium.

The following photomicrographs fail to demonstrate this.

The patient, B—124/91, had her last normal catamenia in April, 1933. Since then she flowed every one to three weeks, using 2-3 napkins each day. She flowed from April 19 of this year to May 6, using six napkins each day. Again she flowed on May 18, and on May 19 a specimen of menstruating secreting endometrium was obtained (Fig. 1). She was given 500 r.u. of Antuitrin-S on May 19, 21, 23, and 25. The flow dimin-

ished on the twenty-second, three days after the first injection, and stopped two days later. On the twenty-eighth, nine days after treatment was started and the first specimen obtained, and three days after the last injection, another specimen was taken (Fig. 2) which was of normal 6-8-day proliferating endometrium. There was no evidence of corpus luteum formation. Again, rather, we have evidence of a rapid regression of the stimulating focus in the ovary. This might be due to the P. U. extract, but it may also be argued that this was to be a normal six-day period anyway, and that the Antuitrin-S had no effect on it. It is still true, however, that there is no evidence of corpus luteum effect after Antuitrin-S.

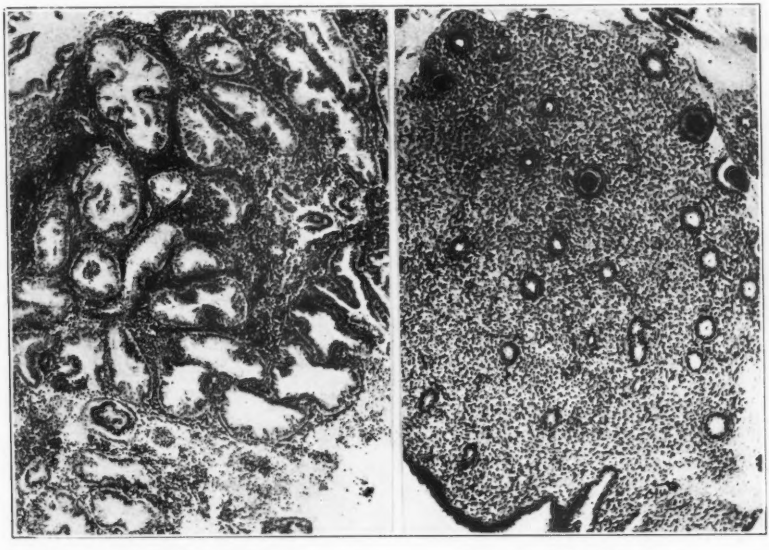

Fig. I Fig. II

Figure I. B—124/91. Intermittent metrorrhagia for one year. Suction specimen taken on second day of flowing after intermission of 12 days. Menstruating secreting endometrium.

Figure II. B—124/91. Metrorrhagia—Three days after last of divided dose totaling 2000 r.u. of Antruitrin-S. Six to eight day proliferating endometrium. No evidence of luteinization.

K—101/118 was 38 years old. Until her present illness her catamenia was regular, every twenty-eight days, lasting four days, requiring five to eight napkins a day. She flowed frequently during the whole of February and March, 1934, and for the first five days in April.

Figure 3 is of endometrium obtained during the active flow on April 5. This shows a dysplasia of the late proliferative type. The glands are many and variously dilated, with closely packed high columnar epithelium. The stroma is dense and of small cells without visible cytoplasm.

The patient was given 200 r.u. of Antuitrin-S on April 8, 9, and 10. On April 10 the flow stopped. A specimen taken on April 16 showed normal very early proliferative or resting endometrium (Fig. 4). If

luteinization had been brought about progestin must have accomplished all its work on the endometrium in eight days. For a progestational endometrium to develop in the first three days of treatment while flowing persisted, and completely to disappear without flow during the next five days is quite unlike the behavior of normal tissue. It is more likely that the P. U. extract caused a rapid diminution of estrin by regression of the follicles which were producing it.

Note that in one case the flow was from a functioning or progestin-stimulated endometrium, whereas the flow from the other was from one

Fig. III

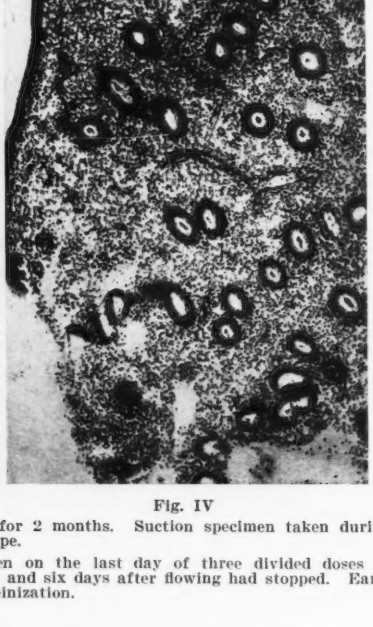

Fig. IV

Figure III. K—101/118. Metrorrhagia for 2 months. Suction specimen taken during active flow. Dysplasia of late proliferative type.

Figure IV. K—101/118. Specimen taken on the last day of three divided doses of Antruitrin-S, totaling 600 r.u. of Antruitrin-S, and six days after flowing had stopped. Early proliferative endometrium. No evidence of luteinization.

which showed only an estrin influence. Is there not a bleeding factor, one which is associated with estrin or with both the hormones, yet one which is obviously influenced by P. U. extract?

COMMENT

Engle as well as Diddle and Allen have shown that P. U. does not stimulate the ovaries of the immature macaque, but Engle also points out that neither does this monkey excrete it in the urine, even in pregnancy. The analogy to humans cannot be too closely drawn. Furthermore, it has been shown by Collip, and also by Leonard and Smith, and by others, that P. U. is more effective on the ovaries of the laboratory animals whose

pituitaries are present than in hypophysectomized animals. It might be expected, therefore, that in women with dysfunctional flow, P. U. might effect follicle stimulation for certain cells of their pituitaries at least must be active, as witness the well-known cystic condition of the ovaries common in such women. Also, Mazer and Katz saw results with a combination of P. U. and the anterior-pituitary growth hormone in female hypoplasia which might again warrant the assumption that, in the presence of an active pituitary, P. U. would be likely to stimulate the ovaries of humans with dysfunctional flow. This may possibly occur and account in some strange way for the prolonged relief from excessive flow which P. U. injections frequently give these patients. There is yet no evidence of this. Geist, on the other hand, reports degenerative changes in the follicles of ovaries removed at operation from patients to whom urinary prolan had been given. These changes were not unlike those produced by Engle in monkeys. It seems that the endometria of the two patients presented here likewise indicate a regression of the ovary from the follicular activity it showed at the time of the flow and before P. U. was given.

Is it not possible that these patients with the well-known polycystic ovaries of dysfunctional bleeding, behave like rodents whose ovaries have been stimulated by earlier injections of P. U. extract? Leonard and Smith found that if such animals are given further injections of P. U. the previously enlarged ovaries of many of them regress.

In these patients it seems probable that regression of the follicles starts immediately with P. U. injections. This removes from the endometrium the stimulation to dysplasia and permits it to return to one of its normal phases. The bleeding is possibly stopped by the direct action of P. U. on the anterior-pituitary bleeding factor, or indirectly by causing a diminution in estrin which in turn affects this same theoretical factor.

SUMMARY AND CONCLUSIONS

The suction curette is a helpful instrument and the endometrium it obtains is an accurate indicator of ovarian activity.

In three patients with long standing amenorrhoea 40 rabbit units of Proluton (Schering's preparation of progestin) caused demonstrable progestational changes in endometrium previously made to proliferate with huge doses of Progynon. It is assumed that if luteinization takes place after P. U. extract, progestin will be formed and its effect seen in the endometrium.

Because the flow in two cases of dysfunctional uterine haemorrhage ceased promptly after injections of P. U. extract, and before adequate luteinization could take place, and because several days after the injections were started and the flow stopped, no evidence of progestin effect could be found in the endometrium, it is probable that some other mechanism than luteinization is responsible.

It seems possible that the immediate effect may be that of P. U. on Novak's "bleeding factor." This primary cessation of the abnormal flow,

however, as well as the frequent failure of such flow to recur, after urinary prolan, may possibly be due to degeneration of follicles such as occurs in normal monkeys, similarly treated.

Addendum: When this paper was originally presented the author was inexcusably unaware of a report by Klinger and Burch (1933). These investigators were led from the use of smaller doses of Antuitrin-S in two patients with dysfunctional uterine hemorrhage to conclusions similar to those of this paper.

REFERENCES

Collip, J. B., H. Selye, E. M. Anderson and D. L. Thomson: Production of estrus. J. A. M. A. **101:** 1553. 1933.

Diddle, A. E. and E. Allen: Reactions of immature monkeys to injections of extracts of pregnancy urine. Anat. Rec. (Supplement). **55:** 54. 1933.

Engle, E. T.: Biological differences in response of the female macacus monkey to extracts of the anterior pituitary and of human pregnancy urine. Am. J. Physiol. **106:** 145. 1933.

Geist, S. H.: Reaction of the mature human ovary to Antuitrin-S. Am. J. Obst. & Gynec. **26:** 588. 1933.

Kaufmann, C.: Die Behandlung der Amenorrhöe mit hohen Dosen der Ovarialhormone. Klin. Wchnschr. **12:** 1557. 1933.

————: Umwandlung der Uterusschleimhaut einer kastrierten Frau aus dem atrophischen Stadium in das der sekretorischen Funktions durch Ovarialhormone. Zentralbl. f. Gynäk. **56:** 2058. 1932.

————: Echte Menstruation bei einer kastrierten Frau durch Zufuhr von Ovarialhormone. Zentralbl. f. Gynäk. **57:** 42. 1933.

Klingler, H. H. and J. C. Burch: Suction in obtaining endometrial biopsies. J. A. M. A. **99:** 559. 1932.

————: The effect of extracts of the urine of pregnant women on the hyperplastic endometrium. Am. J. Obstet. & Gyn. **24:** 17. 1933.

Leonard, S. L. and P. E. Smith: Responses of the reproductive system of hypophysectomized rats to injections of pregnancy urine extracts. II. The female. Anat. Rec. **58:** 175. 1934.

Leonard, S. L.: Differential effect of prolan in decreasing the potency of the hypophysis in normal and castrate rats. Anat. Rec. **57:** 45. 1933.

Mazer, C. and B. R. Katz: Clinical evaluation of combined prolan and anterior pituitary therapy. Endocrinology, **17:** 709. 1933.

Rock, John: Artificial menstruation. New England J. Med. **210:** 1303. 1934.

Werner, A. A. and W. D. Collier: The effect of theelin injections on the castrated woman. J. A. M. A. **100:** 633. 1933.

DIABETES INSIPIDUS: TREATMENT WITH INTERMEDIN AND PITMELANIN*†

PRELIMINARY REPORT OF FIVE CASES

HENRY H. TURNER, M.D.

From the Department of Medicine, University of Oklahoma School of Medicine and the Medical Service of the University and Children's Hospitals

OKLAHOMA CITY

The treatment of diabetes insipidus presents many difficult problems since pituitrin, one of the most effective agents, often has undesirable secondary reactions. Its pressor and gastro-intestinal effects are frequently very distressing to the patient. It has been considered probable that the anti-diuretic action of pituitrin is due to a separate entity (1). Kamm and his co-workers (2) succeeded in obtaining two fractions, the oxytocic and vasopressor, having separate and distinct effects. The pressor fraction (pitressin, vasopressin, B-Hypophyamine), in addition to its effect on the blood pressure, has anti-diuretic properties, and produces an expansion of the melanophores of amphibians. Rowe (3) demonstrated the presence of the melanophoric active substance in association with the vasopressin fraction, and expressed the opinion that it might be a separate principle, and I am informed by Kamm‡ that he has now obtained the pressor principle of the posterior lobe free of melanophore activity. Knaus, Dreyer, and Clark (4) presented further evidence that "the melanophore principle is distinct from the oxytocic and pressor principles."

In 1932, Zondek and Krohn (5) reported the isolation of a pituitary hormone, which they named Intermedin. This substance, according to these workers, has the melanophoric activity of pituitary extracts without the other effects. It has no influence on growth, metabolism, nor on the sexual organs; it does not affect the smooth musculature of the uterus, bladder, and intestines. It does not produce a hyperglycemia nor raise the blood pressure, as do pituitary extracts containing the pressor principle. The distribution of this substance is different from that of oxytocin or vasopressin in the gland and surrounding tissues. It is not present in the sub-occipital liquor where the other two are found, but is present in the colloid of the gland free from oxytocic and pressor substances. By various chemical and physical procedures, Zondek was able to produce an extract free from the other hormones of the pituitary gland, which retained the specific melanophoric effect on Phoxinus laevis.

*I am indebted to Dr. Oliver Kamm of the Research Laboratories of Parke, Davis & Co. for supplying me with the necessary amount of these substances for this study. The Intermedin was prepared according to the method published by Zondek (5). Pitmelanin is the name given by Parke, Davis & Co. to the melanophore principle obtainable from the posterior pituitary, and is not claimed to possess anti-diuretic properties. These products are not yet commercially available.

†Read before the Eighteenth Annual Meeting of the Association for the Study of Internal Secretions, Cleveland, Ohio, June 12, 1934.

‡Personal communication.

The first report of the use of this hormone in diabetes insipidus was by Sulzberger (6) in 1933. His attention was drawn to it by the fact that "it was to be found in the pituitary gland, in the infundibular stalk, and in the walls of the third ventricle," and "these parts of the brain include those in which the lesions in diabetes insipidus are considered to be localized." He tested the effects on a normal individual, and confirmed Zondek as to the lack of undesirable actions. The two cases of diabetes insipidus, which he reports, responded favorably to large doses of Intermedin without any of the unfavorable secondary effects of pituitary extract. It, therefore, seemed to be a therapeutic agent of value in this condition. I have used Intermedin in five cases and Pitmelanin in one case of diabetes insipidus, which are herewith reported.

CASE REPORTS

Case 1 (7). A school boy, aged 13 years, was first admitted to the Children's Hospital in November, 1929, complaining of excessive thirst, marked frequency of urination, a voracious appetite, and overweight. His family history was essentially irrelevant. The parents stated that he had always drunk an excessive amount of water, and had always been hungry, but that these symptoms increased following whooping-cough at the age of 6 years.

The physical examination revealed a well-developed youngster of 9 years, whose height was 52 inches (116 cms.), and weight 105 pounds (48 kgs.). He was approximately 54 per cent overweight. The panniculus was rather generalized, but definitely increased over the breasts, abdomen, and mons veneris. There was a moderate hypoplasia of the genitals. His general appearance was that of dystrophia adiposogenitalis. His average fluid intake for twenty-four hours was 10,800 cc. and daily output 10,530 cc. He was given pituitrin* intramuscularly in increasing daily dosage without restriction of diet or fluids. His intake and output lessened to about 5,000 cc. after two days and to 1,400 cc. on the eighteenth day. The total maximum dose of pituitrin given in twenty-four hours was 74 minims, which produced vasomotor and intestinal reactions. The dosage was gradually reduced to 20 minims daily, which controlled his symptoms. Later, because of suggestive myxoedematous pads and dry skin, and because of the neurohypophyseal syndrome, antuitrin, 1 cc. daily, and thyroid, 5 grains daily, were added. He was discharged from the hospital February 23, 1930, completely relieved of all symptoms, and weighed 84 pounds, a loss of 21 pounds since admission. After returning home he took no pituitrin for a few days, and his original symptoms returned. He was then given 1 cc. pituitrin daily at bedtime, which gave him relief.

On August 28, 1933, he was re-admitted to the hospital, at which time his parents advised that he had taken pituitrin intermittently during the last four years, but none during the past eight months. The polydipsia and polyuria had returned. His weight was 145 pounds, a gain of 60 pounds the past four years, and his height was 61 inches. The obesity was more evident, and he presented a typical picture of Fröhlich's syndrome.

The neurological findings were normal, and perimetric tests revealed no disturbance of the visual fields. Stereoscopic roentgenograms of his skull showed no abnormalities of the sella turcica nor evidence of intra-

*The pituitrin used in this series of cases was Pituitrin "S" (surgical), Parke, Davis & Co.

cranial pressure. The blood count was normal, and there were no pathological cells. The Wassermann test was negative. Total serum protein was 7.3; serum albumen, 2.9; serum globulin, 4.4; serum calcium, 10.8 mg. per cent; serum phosphorous, 4.6; blood cholesterol 220 mg. per cent. The sugar tolerance test was 0.100; 0.219; 0.166; 0.117 per cent, respectively. The urine was clear, specific gravity of 1.002, and free from abnormal constituents. The volume for twenty-four hours was 11,420 cc. The basal metabolic rate was minus 19.1 per cent.

In order to determine the efficiency of various anti-diuretics, especially the pituitary preparations, Intermedin and Pitmelanin, he was placed under observation without restriction of diet or fluid intake (see Fig. 1 for comparative effects). His average twenty-four hour intake over a four-day test period without restriction was 11,600 cc., and average urinary output over the same period, 10,720 cc. His average frequency of intake was 21.2 times, and output 15.5 times. Because of the recent report by Kahn (8), he was given Amidopyrine, grains 10 three times daily, for two days without any apparent effect. The dosage was then increased to grains 15 three times daily during the next two days, and, though there was no change in the total intake and output, there was, according to the patient,

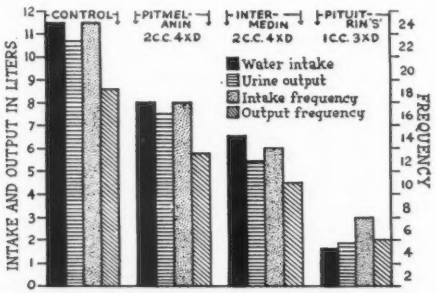

Figure 1. Comparative effects of hypodermic injections of pituitary preparations on water balance and frequency in diabetes insipidus. Each column represents an average of 4 consecutive 24-hour periods.

some decrease in frequency. This, however, was not recorded. He complained of a severe headache while taking such large doses. After four days without treatment Pitmelanin, ½ cc. twice daily, was given for two days without noticeable effect. This was then increased to 2 cc. four times daily for four days with reduction of approximately 3,000 cc. in his intake and output and definite reduction in frequency. After another four-day rest period he was given an equal amount of Intermedin (2 cc. four times daily for four days) with average reduction of his intake to 6,500 cc. and output to 5,400 cc. in twenty-four hours. The frequency and volume were, also, materially reduced, as shown in Figure 1. After another rest period of four days without restriction of fluids and without treatment, he was given 1 cc. pituitrin subcutaneously at 6:00 A.M., 12:00 noon, and 6:00 P.M., which reduced his intake to 1,800 cc., the output to 1,925 cc., and frequency of intake to eight times, and output to six times, in twenty-four hours, with no nocturnal voidings. He was discharged from the hospital on October 8th with instructions to take 1 cc. of pituitrin morning and evening. He had gained 11 pounds in weight.

Each 1 cc. injection of pituitrin was followed by slight nausea, intestinal cramps, and vasomotor reactions, lasting from fifteen to thirty min-

utes, and which made the patient very uncomfortable. No such reactions were evidenced from Pitmelanin nor Intermedin. On the contrary, the patient expressed a feeling of increased well-being, and stated he "wished he could take Intermedin continuously."

Case 2. A female, aged 32 months, had polydipsia and polyuria since birth. The mother stated that these symptoms had increased to the point at which the child was now drinking approximately two gallons of water daily and passing an equal amount of urine. The pregnancy was full term, and the delivery was normal. She weighed 6 pounds 10 ounces at birth; teethed at 6 months; walked and talked at 12 months. This was the second child; a sister, five years her senior, was apparently normal. The patient had had measles at 5½ months, and had always been subject to frequent common colds. She was a difficult feeder and, according to the mother, had never taken any solid food. She drank only one pint of milk daily, and preferred water to ice cream, candy, or anything else. She begged continuously for water during the day, and drank on an average of four to five tumblerfuls during the night. At the age of one year enuresis stopped, but began again at 16 months with increased severity.

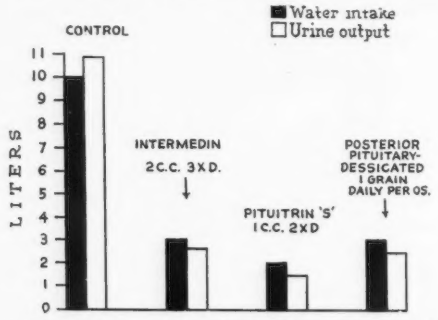

Figure 2. Comparative effects of hypodermic injections of pituitary preparations on water balance in diabetes insipidus. Each column represents an average of 6 consecutive 24-hour periods.

Examination revealed a definitely undernourished youngster weighing 25 pounds, whose height was 36 inches, which was average. She was very difficult to control during examination, crying almost continuously for water and begging to go to the toilet. There was a pallor, and the skin was somewhat clammy, cold, and acrocyanotic. The posterior cervical lymph glands were palpable. The examination otherwise gave negative findings. Stereoscopic roentgenograms of the skull revealed no abnormalities. There was a very mild secondary anemia. Several attempts were made to obtain blood for serologic and chemical analyses without results. The specific gravity of the urine was 1.004; there was no albumen nor sugar.

She was placed under observation for six-day periods, and the average total twenty-four-hour intake was accurately measured at 10,000 cc. The output of 10,800 cc. could not be accurately measured during the night because of the enuresis, but was estimated by comparing the day intake and output. She was given 2 cc. of Intermedin hypodermically three times daily at 6:00 A.M., 12:00 noon, and 6:00 P.M., which remarkably reduced her intake and output approximately two-thirds. After a six-day rest period pituitrin, 1 cc. hypodermically, at 8:00 A.M. and 6:00 P.M., was

given, which further reduced the average intake and output to 2,000 and 1,500 cc., respectively. Blanching of the skin and occasional vomiting followed the latter injections. The pituitrin was continued at this dosage for several weeks, but, because of the fact that smaller doses did not seem to control her symptoms, and because of the reactions produced by the larger doses, and extreme difficulty in giving her hypodermic injections, the parents insisted that it be discontinued. They advised that they had recently heard of a new treatment, which could be given by mouth, and suggested trying it. She was, therefore, given Elixir Pyramidon, 1 teaspoonful four times daily by mouth, which had no effect. On further insistence of the parents for oral therapy, 1/10 grain desiccated posterior pituitary (Parke, Davis & Co. Emplets), 2 tablets three times daily, was prescribed. There was some apparent lessening of the symptoms; therefore, the dosage was increased to a total of 1 1/5 grains daily, and the intake and output again measured over a six-day period. The results were comparable to those of Intermedin, i.e., intake 3,000 and output 2,300 cc. in twenty-four hours. Some time later pituitrin by intranasal spray and insufflation of pituitary powder were tried with good results, but soon dis-

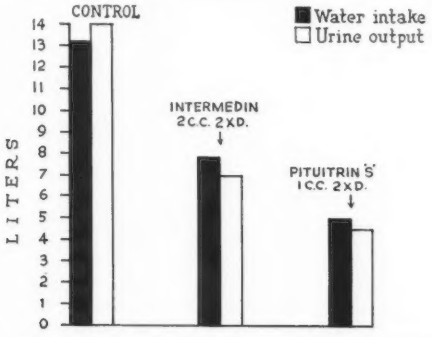

Figure 3. Comparative effects of hypodermic injections of pituitary preparations on water balance in diabetes insipidus. Each column represents an average of 10 consecutive 24-hour periods.

continued because of objections of the patient. She has been taking 1 grain desiccated posterior pituitary daily during the past five months with marked relief of her symptoms. Her appetite has improved; she has gained 5 pounds in weight; and sleeps all night without awakening and without enuresis (Fig. 2).

Case 3. A male, aged 26 years, was first admitted to the University Hospital in 1925. He complained of extreme thirst, excessive urination, nervousness, and loss of appetite and weight. Two years previously he had had an acute encephalitis, and his present symptoms appeared six weeks later. His fluid intake varied from 15 to 28 liters during each twenty-four hours, and the urinary excretion exceeded the water intake by several hundred cubic centimeters. His excessive thirst and frequency prevented proper rest. He had lost 32 pounds in weight in twenty months, and was greatly fatigued.

Examination in 1925 revealed a tall, thin, obviously nervous boy of 18 years. He weighed 138 pounds (63 kg.), and his height was 70 inches (175 cm.). His face was mask-like, and there was a slowness of all voluntary movements, a fine tremor of the tongue, and coarse intentional tremors

of the limbs. The deep reflexes were somewhat exaggerated, and there were suggestive pathological toe signs on the right. His memory was fairly good, but consciousness was somewhat clouded. He had some disturbance of perception with delusions of persecution. When unrestricted his intake and output averaged 22 liters daily.

He was given whole gland pituitary substance (desiccated), 1 grain daily by mouth, without effect. Later he was given 1 cc. pituitrin, and immediately complained of severe headache and nausea. His systolic blood pressure rose 52 points, he became unconscious, and convulsions occurred, which were followed by deep sleep for about four hours. Smaller doses of ½ cc. of pituitrin were then given twice daily, which gave mild reactions, but reduced his water exchange approximately fifty per cent. He was discharged with instructions to keep up this medication.

He returned to the hospital several times during the following three years for observation, and his symptoms were approximately the same as before. He continued his medication only a few days following each visit to the hospital.

Upon re-admittance September 1, 1933, he stated that he had been very well since his last visit in 1927, except for some increased tonicity of

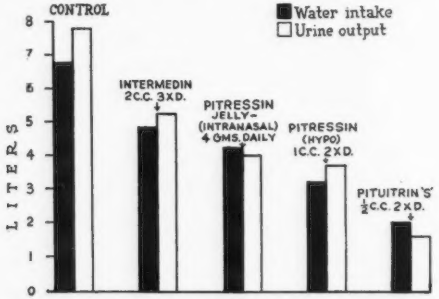

Figure 4. Comparative effects of hypodermic injections of pituitary preparations on water balance in diabetes insipidus. Each column represents an average of 10 consecutive 24-hour periods.

his muscles and occasional oculogyric crises. His thirst and urinary output continued to be excessive. He had taken no pituitary preparations during the past six years. At this time his unrestricted water intake over a ten-day period averaged 13.2 liters daily and his urine volume 14 liters (Fig. 3). Amidopyrine, grains 15 at bedtime for three consecutive evenings, had no appreciable effect. This was increased to grains 15 three times daily, and he complained bitterly of headache and nausea. There was no reduction in the fluid intake, frequency, nor total output. Following a three-day unrestricted period, he was given Intermedin, 2 cc. hypodermically, at 6:00 A.M., 12:00 noon, and 6:00 P.M. Within three hours it was observed that he was taking less water, and during the ten-day period the intake was reduced to 8 liters and excretion to 7 liters during each twenty-four hours. He stated that he felt very comfortable, and there were no untoward reactions of any sort noted. Pituitrin, 1 cc. twice daily, was then given, which caused further reduction in his intake to 5,000 cc. and output to 4,500 cc., but he complained of severe headaches, nausea, vomiting, and became very apprehensive following each injection. Because of the excellent results obtained in other cases of post-encephalitic Parkinson syndrome, I desired to establish his tolerance to Atropine and

observe its effect upon the symptoms of diabetes insipidus, but he refused to remain longer in the hospital.

The laboratory findings in this case showed the skull to be normal. Blood chemistry values were all within normal limits. The fasting blood sugar was 84.2 mg. The blood Wassermann findings were negative.

Case 4 was that of a white male, aged 38 years, who stated that he had had an unsatiable thirst with marked frequency of micturation during the past sixteen years. There was no history of any severe illness nor injury. He drank from 3 to 6 ounces of water or some soft drink approximately every thirty minutes, and 3 to 4 large glassfuls during each meal. If excited or worried, he often drank every ten minutes during the day, and found it necessary to get up 5 or 6 times during the night. He had a very voracious appetite, but ate moderately. He had had a craving for sweets for several years, and used them rather excessively—6 to 8 spoonfuls of sugar on a half grapefruit, etc. At the age of 25 years he began gaining weight, and gained 40 pounds in two years, but his weight had remained rather constant since then. He had noticed increasing fatigue

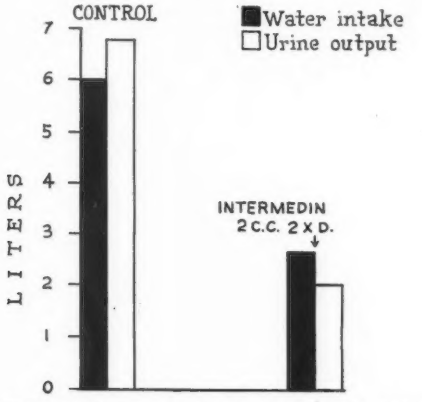

Figure 5. Comparative effects of hypodermic injections of pituitary preparations on water balance in diabetes insipidus. Each column represents an average of 6 consecutive 24-hour periods.

and irritability in the past 5 or 6 years. His sexual powers had decreased to almost nil within the past few months.

Examination revealed a very well-developed, well-nourished male, whose weight was 190 pounds (86.5 kg.), and height 71¼ inches (178 cm.). There were no remarkable findings, except that his features were somewhat acromegalic, although there were no other evidences of this condition. His blood pressure was 130/90, and the pulse rate 90 per minute. Ophthalmoscopic examination revealed normal fundi. Stereoroentgenograms revealed no distortion nor evidence of erosion in the region of the sella turcica. The frontal sinuses were very large. The blood picture was normal. The basal metabolic rate was minus 31. The blood Wassermann findings were negative. The sugar tolerance test gave the following: fasting specimen, 105 mg. per 100 cc. of blood; 45 minutes later, 152 mg.; two hours later, 98 mg.; there was a faint trace of sugar in the urine at the end of the 45-minute period, but the second hour specimen was normal. His average twenty-four-hour intake was 7,000 cc., and his output 7,800 cc. (Fig. 4).

Pitressin jelly, 1½ grams intranasally, was prescribed. The effect was so slight that the dosage was increased to 6 grams daily, which reduced his total water consumption to 4,300 cc., and excretion to 4,000 cc. daily. He complained that the jelly produced a burning and swelling of his nasal mucous membranes, which interfered with breathing. After a five-day rest period, pitressin, 1 cc. twice daily hypodermically, was prescribed. This reduced his intake to 3,300 cc. and output to 3,500 cc. He complained of weakness and nausea after each injection, and there was some blanching of the skin. These symptoms did not appear if the hypodermics were given immediately after a meal. After another rest period, pituitrin, ½ cc., was given hypodermically, which reduced the intake to 2,000 cc., and the output to 1,800 cc. Mild vasomotor reactions were noticed with some nausea, and he complained of a feeling of extreme depression for about one hour after each injection. Nasal insufflations of pituitrin were next tried, ½ cc. in each nostril, but, because of an extreme burning sensation and nasal blocking, he refused to continue this treatment after the second insufflation. After a rest period of about two weeks, during which time his intake and output returned to the unrestricted level, he was given Intermedin hypodermically, 2 cc. three times daily over a ten-day period, with reduction of his average daily intake to 4,900 cc. and his output to 5,200 cc. Although the Intermedin was not as effective, and did not reduce his thirst and diuresis to the extent of the other pituitary preparations, he stated that he felt much more comfortable during the time he was taking it. No untoward reactions of any sort were experienced, and he stated that his thirst and frequency were almost unnoticeable and that he was able to sleep soundly throughout the night.

Case 5. A male, aged 24 years, complained of polydipsia and polyuria. In an automobile accident six months previously he had sustained a basal fracture of the skull, and was unconscious for five days following the accident. He had a Horner syndrome and analgesia of the left face, both of which had markedly improved. The symptoms of diabetes insipidus appeared approximately two weeks after the accident. His physician at that time gave him pituitrin, 2 cc. one day, followed by 1 cc. the next, with a rest period of 2 to 3 days in between, which, he stated, had no effect on his symptoms, and was discontinued because of headaches and severe abdominal cramps. He believed that there was some gradual lessening of his intake and output during the past six months; however, they were still of sufficient severity to inconvenience him. He was very nervous, easily upset, and emotionally unstable. There had been a complete loss of libido since the accident.

Examination revealed a tall, well-developed individual, whose weight was 158 pounds (72 kg.), and height 71 inches (177 cm.). He was of the non-obese eunuchoid type, with rather sharp features and scant beard. He stated that he shaved only twice a week. There was no hair on the chest, abdomen, nor extremities, and the pubic triangle was feminine. The genitals were moderately well-developed. The right pupil was somewhat irregular, but reacted to light and accommodation, and there was no ptosis of the lid. The eye muscles were intact. Ophthalmoscopic examination revealed no abnormalities of the fundi, and perimetric examination revealed no constriction of the visual fields. The blood pressure was 110/70, and the pulse rate 80 per minute. Superficial and deep reflexes were present and equal. His daily intake and output over a ten-day period averaged 6,000 and 6,800 cc., respectively. He was given 2 cc. of Inter-

medin hypodermically three times daily with very marked results. The intake was reduced to 2,500 cc., and the output to 2,000 cc. daily (Fig. 5).

SUMMARY

Comparative effects of Intermedin, Pitmelanin, and other pituitary preparations on the water balance in five cases of diabetes insipidus were studied. A reduction in diuresis and thirst occurred in all patients after the administration of intermedin, though the response was not so great as that obtained from equal amounts of pituitrin. Because of their freedom from pressor effects, the patients preferred intermedin and pitmelanin to other pituitary preparations. In one case symptoms were controlled by the oral administration of posterior-pituitary substance. Intermedin and pitmelanin contain the antidiuretic factors of the hypophysis, and are of value in controlling the symptoms of diabetes insipidus.

REFERENCES

1. Dudley, H. W.: Some observations on the active principles of the pituitary gland. J. Pharmacol. & Exper. Therap. **14**: 295. 1919-20.
2. Kamm, O., T. B. Aldrich, I. W. Grote, L. W. Rowe and E. P. Bugbee: The active principles of the posterior lobe of the pituitary gland. I. The demonstration of the presence of two active principles. II. The separation of the two principles and their concentration in the form of potent solid preparations. J. Am. Chem. Soc. **50**: 573. 1928.
3. Rowe, L. W.: Studies of oxytocin and vasopressin: The effect on frog melanophores. Endocrinology, **12**: 663. 1928.
4. Knaus, H. H., N. B. Dreyer and A. J. Clark: A note on the melanophore dilator action of the pituitary. J. Physiol. **60**: Proc. of the Physiol. Soc., XVIII. 1925.
5. Zondek, B. and H. Krohn: Harmon des Zwischenlappens der Hypophyse (Intermedin). III. Zur Chemie, Darstellung und Biologie des Intermedins. Klin. Wchnschr. **11**: 1293. 1932.
6. Sulzberger, M. B.: The pituitary hormone intermedin as the active anti-diuretic in the treatment of diabetes insipidus. J. A. M. A. **100**: 1928. 1933.
7. Turner, H.: Endocrine clinic: diabetes insipidus; Laurence-Biedl syndrome. J. Oklahoma M. A. **24**: 148. 1931.
8. Kahn, B. S.: The use of amidopyrine in a case of diabetes insipidus. J. A. M. A. **100**: 1593. 1933.
9. Gibbs, O. S.: A practical test for the anti-diuretic action of pituitary. J. Pharmacol. & Exper. Therap. **40**: 129. 1930.

SUCCESSFUL OVARIAN THERAPY IN A CASE OF SEVERE PREADOLESCENT EUNUCHOIDISM

H. LISSER, M.D.
SAN FRANCISCO

Even though experimental investigations increasingly demonstrate manifold interglandular relationships, and future research unravels some of these pluriglandular complexities, sound clinical practice should attempt to recognize the uniglandular origin of multiglandular syndromes, and focus treatment on the ailing organ primarily responsible. The recent revelations that the anterior pituitary, in addition to growth- and mammary-stimulating hormones, manufactures thyreotropic, adrenaltropic, contrainsular and gonadotropic hormones, however fascinating and important, will not alter specific thyroid therapy for myxedema, adrenal cortical extract for Addison's disease, insulin for diabetes mellitus, and ovarian hormones for primary ovarian deficiencies. And amidst the flutter over pituitary-gonadal rhythms it would be well to remember adrenal-gonadal and thyroid-ovarian intimacies, as in adrenal-cortical amenorrhoea and hypothyroid menorrhagia. And however much the dependent gonads must pay tribute to their "motor," the pituitary, at least let it be recognized that the ovaries can start trouble on their own account and be initially responsible for syndromes of their own. Castrate patients, then what have you?

With no intent to disparage the illuminating light being shed on endocrine interrelationships—quite the contrary, it should be emphasized that clinical efforts must aim to keep the horse before the cart, and to feed the horse if he needs food, and not the cart.

All of which is introductory to recalling that von Tandler and Gross, many years ago, published classical descriptions of the appearance presented by individuals who had been castrated in childhood—preadolescent eunuchism. It has been recognized since that time that there are encountered now and again, persons who conform in almost every detail to the castrated victims above referred to, but who have never been castrated. Their condition was, quite reasonably, interpreted as a severe deficiency of gonadal function originating prior to puberty and therefore termed preadolescent eunuchoidism.

Briefly epitomized, a frankly pronounced case of preadolescent eunuchoidism will display the following characteristics: (1) disproportionate tallness without actual gigantism; long extremities, so that the "lower measurement" (symphysis pubis to soles of feet) markedly exceeds the "upper measurement" (symphysis to vertex), and the span is greater than the height; long slender fingers and long narrow feet; (2) retarded bone-age due to delayed closure of the epiphyses; (3) incomplete develop-

ment and late appearance of the secondary characters of sex. In the female the latter is manifested by absence or late onset of menstruation, infantile uterus, lack of mammary enlargement, sparse or absent pubic and axillary hair—at an age of 16 years or over.

The above mentioned characteristics may occur in association with two diverse types of body configuration; (a) the markedly obese type and (b) the thin undernourished, enteroptotic constitution sometimes associated with chlorotic anemia, meager vitality, and corresponding neurasthenic psyche.

Now if one is confronted with a tall, slender girl of seventeen or thereabouts, with dangling arms and legs, lacking breast development and showing perhaps a few wisps of pubic hair, and one is told that she has never menstruated, and a rectal examination reveals a tiny uterus—the diagnosis is plain; it should be obvious that the therapy required should be that which attempts to compensate for the lacking ovarian hormones. It is the successful application of this simple principle which constitutes the excuse for publishing a single case report, with an added reason—emphasis on patience and persistence.

CASE HISTORY

The patient was 17 years and 3 months old when first seen in May, 1931, kindly referred by Dr. Donald J. Frick, of Los Angeles, California.

To conserve space it may be stated bluntly that her "past history" contained nothing which could be considered as having any bearing on the condition for which she sought advice.

Familial and hereditary factors, however, could not be so lightly disregarded. Her father admitted that he had, perhaps, been "a little late" in maturing. Her mother was not certain as to the precise age when she first menstruated but thought it occurred at 15 years of age.

Her brother consulted us at the age of 21 years; his puberty had been somewhat delayed and shaving was necessary but once a week; however, his voice was of normal adult pitch, pubic and axillary hair was abundant, and the external genitalia were thoroughly satisfactory and of full adult dimensions.

Her sister consulted us in July, 1932, when she was 15½ years of age; menstruation had not appeared and although the secondary sex characteristics were slightly retarded, they were far advanced over the status to be described for her sister two years older. Her pubic hair was a little sparse for her age, and although the breasts contained ample mammary tissue the nipples and areolae were still somewhat adolescent and therefore backward for her age. Her "bone age" was estimated (by roentgenograms) at 14 years, and her measurements disclosed disproportionately long extremities, the "lower exceeding the upper" by 5½ inches. She was considered to represent a relatively mild but definite example of preadolescent eunuchoidism, probably on a constitutional hereditary basis. Her menstruation began three days after we saw her.

There existed, therefore, a significant family background influencing delayed maturity, which, apparently, reached a climax in the daughter now to be described.

She was a large baby (exact birth weight not known) and a little late in teething, walking, and talking. She was an active child always, but overweight until about 14 years of age, despite participation in athletics. School work had never been easy for her; it was necessary for her to repeat some work in the 9th grade. She was not stupid but had to work hard to get along.

Dr. Frick was consulted when she was 16 years of age. He reported that, other than pronounced sexual immaturity, the findings were essentially normal, except that the blood pressure was low (95 systolic over 70 diastolic). The sella turcica was probably a little small but showed no evidence of erosion, and the skull as a whole was not unusual. The basal metabolism ranged from minus 19 to minus 2, depending on thyroid dosage. The blood count and blood chemistry were normal, and the Wassermann test gave negative findings.

The principal of the private school she attended described her as fearful of her work and very nervous about herself, excitable and sensitive; her nervous confusion evidenced itself often in blankness, complete lack of memory; at other times she grasped a point very readily. "One feels that she understands, that there exists good mentality, but that a physical obstacle intervenes which makes for little control of mind and speech, and at times she is utterly incapable of expressing herself. Socially she is a delightful companion, markedly at ease with older people."

Examination. Our patient was a tall, reticent girl, 5 feet 9⅝ inches in height (without shoes) and weighing 133¾ lbs. (without clothes). The ideal weight for her height and age is 141 lbs. Her body contour was more masculine than feminine. Her span exceeded her height by two inches, and the lower measurement was seven inches greater than the upper; in other words, disproportionately long extremities or eunuchoid proportions. Her hands and feet were rather long and narrow; she wore a size 8-B shoe. She had not menstruated (age 17 years and 3 months).

Axillary hair was barely noticeable and there were just a few wisps of pubic hair. The scalp hair was normal in amount and texture. Her skin was smooth and without acne or abnormal pigmentation.

The breasts were practically flat; it was doubtful whether a little mammary tissue could be palpated. The nipples and areolae were absolutely childish.

The external genitalia, more particularly, the labia, were poorly developed, and corresponded to those of an eight or ten-year-old girl. Only a rudimentary thickening, probably uterus, could be palpated rectally.

Otherwise the physical examination revealed no abnormalities. The blood pressure was 108 over 88; on subsequent occasions it varied from as low as 72 to 100 systolic, over 50 to 70 diastolic.

The routine urine and blood findings were normal except for a preponderance of lymphocytes, 65 per cent.

A roentgenogram of her chest showed no evidence of enlarged thymus.

As was to be expected, roentgenograms of her extremities revealed a "bone-age" of 14 years, a retardation of 3 years.

The basal metabolic rate (on thyroid therapy) was 3 per cent plus.

Diagnosis. In short, we were dealing with a pronounced degree of primary preadolescent eunuchoidism, as evidenced by absence of menstruation, almost complete absence of secondary sex characteristics, infantile internal and external genitalia, plus relative tallness with disproportionately long extremities and retarded bone age in a girl aged 17 years and 3 months.

The prognosis was by no means encouraging but the specific type of therapy and the paramount importance of persistent treatment for several years, was clearly indicated.

Treatment. The following measures were prescribed: Two tablets three times daily of agomensin (Ciba), and 1 cc. or 50 units, subcutaneously, three times a week, of theelin (Parke, Davis & Co.). This ovarian therapy was instituted on May 16, 1931. Thyroid substance sufficient to maintain a normal basal metabolism was continued, one grain daily of the desiccated material. It is to be emphasized that there was no clinical evidence of hypothyroidism and that the slightly low basal rates previously obtained were secondary to the ovarian inactivity. Also, the subsequent improvement under the intensive ovarian therapy cannot be attributed fairly to the incidental thyroid dosage since the latter had been administered for over a year before we saw the patient without noticeably influencing her severe sexual retardation.

Progress. Two weeks later, by June 2, a definite bulging of the left breast with unmistakable development of mammary tissue was clearly noticeable. The nipple areola had undergone a little change also.

A more exact status of the pelvic organs, their size at least, was sought and Dr. William G. Moore, gynecologist, was consulted. His report was:

"*Examination*—Tall, rather thin. Practically no mammary development, although there is a small amount of glandular tissue in left. Pubic hair is very scanty. Labia are poorly developed. Rectal examination shows very small cervix, body not definite but there is sufficient thickening above cervix to suggest uterine tissue. The right ovary is about ¼ normal size, the left slightly smaller. Vaginal examination with endoscope shows vagina about ⅞ cm. in length and cervix less than a cm. in diameter with patent os. *Conclusion*—Retarded development of pelvic organs. It is possible that uterus may develop sufficiently under glandular therapy to bring about menstruation, but it does not seem at all likely."

By July 7, a month later, further development of the left breast was apparent, and the bare beginning of swelling in the right was discernible. The mother volunteered that her daughter seemed quieter, less jumpy, and happier.

In September, at the end of four months' treatment, both breasts were nicely developed and of equal size; there was more axillary hair; and the labia were maturing. All these factors encouraged faithful continuance of the same therapy.

In December, seven months after beginning treatment, Dr. Moore checked the status of the internal genitalia. He could not detect any improvement. He was impressed, however, by the unquestioned mammary development and the increase in axillary and pubic hair.

In April, 1932, progynon, one tablet three times daily, was substituted for agomensin. The theelin dosage remained the same. The injections of the latter three times a week had been faithfully continued.

At the end of a year's treatment the patient's bone age was checked; it was estimated at between 16 and 17 years (the patient's chronological age being 18 years and 3 months), less retardation than at first. Basal metabolic determinations made from time to time were normal. She had grown 1¼ inches and gained 9½ pounds and had reached the proper weight for her height and age. Her figure and contours were normal and attractive.

In June, 1932, one year after her first examination, Dr. Moore was able to report definite improvement: "The cervix has practically doubled in size and I am quite sure that there has been a definite increase in the uterine tissue."

This was most gratifying and encouraged us to persist with treatment.

Her first menstrual period occurred on September 3, 1932, when she was 18½ years old, one year and a little over three months since intensive ovarian therapy was started; a fitting reward for patience and perseverance.

The patient was granted a respite from the intensive therapy over the Christmas holidays. Menstruation had not occurred since September 3, 1932, and on January 13, 1933, injections of antuitrin-S were substituted for theelin with the thought that, perhaps, pituitary stimulation of the ovaries might be helpful. On March 3, A.P.L. (Collip's Anterior-Pituitary-Like fraction) from human placenta was ordered in place of antuitrin-S. Both of these preparations had to be abandoned because of severe local reactions at the site of injection, culminating in two large sterile abscesses.

Uterine bleeding lasting only an hour occurred on April 20, 1933, after which all treatment was discontinued.

In June, 1933, two years after his initial investigation, Dr. Moore was able to report further increase in size of the uterus, though the long cervix was still of the infantile type, and the ovaries small. He added . . . "Considering the improvement in the past years, the outlook is far more favorable than I thought at first."

It was decided to abandon all therapy for the summer months. Scanty menstruation occurred July 5 to 8, 1933. With the hope of consolidating the gains already made and stimulating further advances, progynon, one tablet daily, was begun in September. A thoroughly normal period took place in January, 1934, lasting 5 days, 10th to 15th. Subsequent menstrual periods appeared February 8, March 4, April 14, May 7, May 28, June 23, and July 10. The only medication during this time was the progynon, one tablet daily, which was decreased to one every other day when the periods began to occur too frequently. It is to be noted that no thyroid was given after June, 1933.

It is interesting to record that in June, 1933, the patient graduated with high honors from a private school with severe scholastic standards. It will be recalled that she had experienced considerable difficulty with her studies prior to the intensive ovarian therapy. It is just probable that this treatment had some beneficial influence, at least emotionally. No doubt the striking transformation in her physical appearance as brought about by the development of normal secondary sex characteristics, contributed to her poise and self-confidence. A few months before her mother had written as follows: "We are so pleased and gratified with the great change that we all see in the patient since Christmas holidays. Physically she has made tremendous strides—not only in her actual bodily development, but in her quietness and poise and lack of nervousness. Perhaps even more than this I appreciate the mental awakening which has taken place. She is so much more "on her toes"—interested in many things, very thoughtful about many which she had practically ignored until recently, and much, much less ingrown and self-absorbed. I wonder if you see this as I do, and if you feel that it indicates the other development which we have so eagerly watched for?"

Comment. The critically-minded reader may be disposed to regard the therapeutic effects in this case as strictly coincidental and believe that the patient represents an extreme example of a natural delayed maturity. No on can convincingly contradict such an attitude. Furthermore, a familial background of slightly retarded puberty might be interpreted as supporting such an opinion. Nevertheless, the writer, with every wish to be conservative, thinks otherwise. He regards the patient's severe eunuchoidism as the pathological fulfillment of an hereditary tendency which, untreated, would not have righted itself. He interprets the outcome as a most gratifying example of specific endocrine therapy, properly applied and faithfully continued. Neither contention can be disproved and one is free to make one's choice.

However, the psychological importance of accelerating the onset of puberty, at such a late age, is of the utmost significance. Embarrassment, self-consciousness and a feeling of inferiority are inevitable in a girl or lad of 15 or over when sex characteristics are obviously lacking. They shrink from associating with either sex of their age period, withdraw from activities, seek the more sympathetic shelter of older people and—develop "complexes." The rather objective casual attitude of the physician who blandly reassures the parent that "nature will take care of it" is indication either of ignorance of the fact that nature sometimes doesn't or of cruel indifference to the heartaches of these patients. Even if there be justifiable difference of opinion as to how influential such therapy may be, the patient deserves the benefit of the doubt. There is less harm from treatment than there may be without it.

SUMMARY

The narrative has been related of a girl aged 17 years and 3 months, whose secondary sex characters were entirely absent except for a few wisps of pubic hair, and whose internal genitalia were very severely retarded. Her physical appearance simulated that of a eunuch castrated in childhood. The outlook for normal sexual development seemed gloomy indeed. She was placed on ovarian therapy (agomensin, later progynon, and theelin from the outset) for three years. Secondary sex characters were stimulated fairly promptly and are now entirely normal. After a year and three months, the first menstrual period occurred when the patient was 18½ years old. Several months intervened between the next few periods but now at 20 years of age copious menstruation is occurring every month.

THYROID AND SENESCENCE

STRUCTURAL TRANSFORMATIONS OF THE THYROID IN OLD AGE AND THEIR FUNCTIONAL INTERPRETATION

G. C. DOGLIOTTI AND G. NIZZI NUTI
From the Institute of Medical Pathology of the University of Florence
Professor P. Bastai, Director
FLORENCE, ITALY

It is generally believed that the thyroid of the old becomes hypotrophic and loses its efficiency as do other organs and tissues of the body. This conception, however, is based largely on inconclusive evidence (the presence of conditions often associated with hypothyroidism such as falling

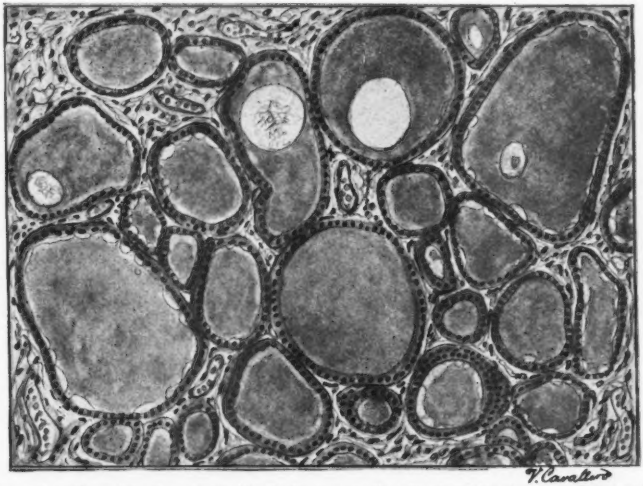

Figure 1. Thyroid of an individual 30 years of age (normal type). Enlargement 200 diameters.

of the hair, dryness and rugosity of the skin and of the nails, and hypothermia). Some workers (Benedict, Horsley and Vermehren) have found a diminution of the basal metabolic rate but this finding has not been confirmed by others (Lucchi). Several authors (Lorand and Gray, Horsley and Vermehren) believe that senescence is in an important measure due to hypothyroidism.

One of the most essential data in favor of this hypothesis is the presence of regressive and degenerative phenomena in the thyroids of the old (Clerc). However, analogous observations have been made by numerous anatomists (Ziegler, Kaufmann, Farrant) on the thyroids of young subjects dying of infectious or exhausting diseases. In view of the possibility that the histological transformations described by Clerc might be ascrib-

able to the disease which caused death and not to senescence "per se," we have re-studied the structure of the thyroids, discarding those cases in which the thyroids themselves were manifestly diseased or were derived from individuals dying of serious infections. These researches are a part of a program of investigations on senescence which have been in progress for several years under the direction of Professor Bastai.

Our investigations have been carried out on more than fifty thyroids collected at autopsy in the Institute of Pathological Anatomy of Florence. Some were imbedded in paraffin, after fixing in formalin, Zenker-formalin or Fleming's solution. In other cases sections were made with a freezing microtome. The sections were stained with haematoxylin-eosin, Mallory-Azan's stain, Weigert's stain for elastic tissue, Daddi's stain for fat and Ciaccio's stain for lipoids.

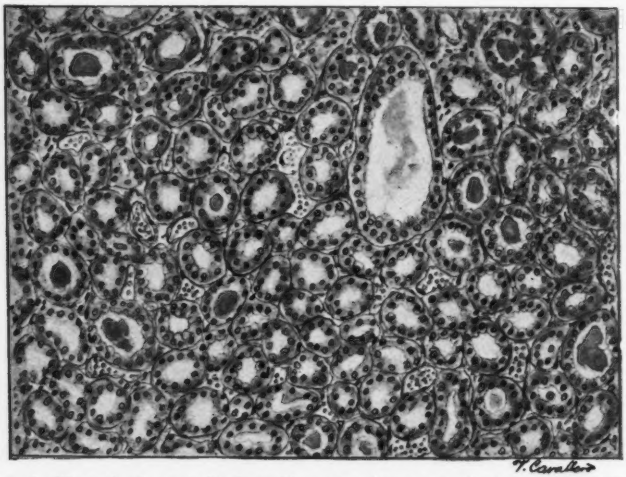

Figure 2. Thyroid of an individual 70 years of age (senile type). Enlargement 200 diameters.

Alterations of regressive or degenerative type, such as described by Clerc in advanced age, have been observed by us at any period of life, when the cause of death was a serious infectious or exhausting disease and macroscopically the thyroid already presented various signs of degeneration (softness or excessive hardness, fragility, yellow or reddish color, etc.).

There are various histological pictures observed in connection with age. During the early years of life the thyroid appears to be made up of very small and losely lying follicles, with relatively large epithelial cells; this fact is particularly evident during the period of puberty, at which time the thyroid also obviously increases in weight. At the other periods of life the weight of the thyroid does not show any consistent tendency to change of weight but it is subject to gradual, profound structural transformations. In middle age the follicles are of various diameters from a few to several hundred millimeters. The epithelial cells are cuboidal and

Figure 3. Thyroid of an individual 80 years of age (senile type). Enlargement 700 diameters.

rather small, or more or less flattened, according to the size of the follicles. The interstitial connective tissue is fairly abundant (Fig. 1). During the presenile period (from 50 to 65 years of age) the percentage of relatively small follicles with but little colloid distinctly increases; during the senile period (over 65 years of age), the follicles are still more reduced in volume, so that their cavity becomes practically obliterated and the colloid disappears from all of them (Fig. 2). Next to these obliterated follicles, which usually constitute the majority, there are found—in quantities which vary from one case to another—very small follicles. But there are,

Figure 4. Thyroid of an individual 75 years of age (senile type). Enlargement 600 diameters.

however, senile thyroids in which colloid no longer exists and the histological aspect of which is totally altered. Under these conditions the epithelial cells are greatly enlarged in volume, of cylindrical or rounded shape, and with protoplasm rich in proteinic secretion granules (Figs. 3 and 4).

The interstitial connective tissue remains unchanged or is slightly increased. The same is true of the elastic tissue which, in the thyroid, is very scarce.

COMMENT

The histologic picture of the senile thyroid shows that this organ functions more actively in normal old age than in earlier adult years. The hyperplasia of the secreting cells, with the increase of secretion granules, indicates an accelerated activity of these elements; the diminution of colloid demonstrates a more intense resorption of the same. These conditions correspond to those which have been observed in states of hyperthyroidism (Basedow's disease, following splenectomy, etc.)

It has been observed (Rössle, G. Levi, etc.) how in old age, together with evidences of regression (sclerosis, atrophy) certain compensatory phenomena arise: an increase of the elastic stroma of the myocardium tends to compensate for diminished contractile efficiency of the myocardial fiber (Dogliotti); the increase of arterial pressure tends to compensate for the increased peripheral (capillary) resistance which, in later years, becomes more and more evident (Bastai). Likewise we consider the senile hyperfunction of the thyroid as a compensatory phenomenon. It affords an additional supply of a notably powerful metabolic stimulant at precisely the time when the activity of the body cells and the exchange between blood and tissues slackens. While such an hypothesis is difficult of direct proof it accords with, and furnishes a *raison d'etre* for the histological phenomena herein reported.

SUMMARY

From histological researches carried out on the thyroid glands of 50 aged subjects it was found that this gland in old age undergoes profound structural modifications. These include reduction of follicles, diminution of the colloid material and hypertrophy of the epithelium. These transformations are indicative of hyperfunction. The augmented thyroid secretion is regarded as a phenomenon of compensation for the senile decline of metabolism.

REFERENCES

Anderson, O. A.: Arch, f. Anat. u. Entwcklngsgesch. **45**: 225. 1894.
Bastai, P.: Minerva med. **2**: 749. 1933.
Bensley, R. R.: Am. J. Anat. **19**: 37; 57. 1916.
Clerc, E.: Frankfurt. Ztschr, f. Path. **10**: 1. 1912.
Cowdry, E. V.: Am. J. Anat. **30**: 25. 1922.
Erdheim, J.: Beitr. z. path. Anat, u. z. allg. Path. **33**: 123. 1903.
Farrant, R.: Lancet **1**: 680. 1914.
Hürthe, K.: Pflüger's Arch. f. d. ges Physiol. **56**: 1. 1894.
Iscovesco, H.: Compt. rend. Soc. de biol. **65**: 84. 1908.
Kraus, E. J.: Virchows Arch. f. path. Anat. **218**: 107; 127. 1914.
Müller, L. R.: Beitr. z. path. Anat. u. z. allg. Path. **19**: 127. 1896.
Schmid, E.: Arch. f. mikr. Anat. **47**: 181. 1896.
Wegelin, C.: Frankfurt, Ztschr. f. Path. **4**: 147. 1912.

THE ANTERIOR PITUITARY HORMONE CONTENT OF THE HYPOTHALAMUS OF DOGS

PROF. DR. GIACOMO PIGHINI
Director of Scientific Laboratories, Psychiatric Institute
REGGIO EMILIA, ITALY

In a previous research (1) it was shown that the tuber cinereum of man contains a hormonal substance similar to the gonadotropic principle of the anterior hypophysis. Phenolated extract of tuber tissue injected into male and female immature rats evoked maturation in the gonads of both sexes. The intensity of reaction was even greater than when hypophyseal extracts were used. Collin and Watrin (2) in part confirmed these findings. They showed that tuber grafts in young guinea pigs in many instances stimulates gonad growth but in others a contrary effect was obtained. That a species factor may be involved in this problem is indicated by my findings that the tuber cinereum of the ox fails to show the hormonal activity that is demonstrable with human tissue.

It was decided, therefore, to repeat the experiment, using dogs as donors. Two groups of three puppies each were used. These were littermates. The animals of one group were treated for 40 days with a phenolated emulsion of the anterior pituitary prepared as previously reported (3) (4).*

The dogs were sacrificed and immediately a block of tissue containing tuber cinereum and weighing from 0.22 to 0.26 grams was cut out. One extract-treated and one normal donor were sacrificed in immediate succession with chloroform. From each was collected the same amount of diencephalic (tuber-containing) substances which was at once emulsified in 10 cc. of phenol solution. Each pair of dogs was similarly treated.

The extracts were injected into 16 immature female rats obtained from two litters born on the same day. Injections were begun on the forty-second day of age, when each rat weighed from 42 to 43 grams. The vulvar orifice was still closed in all cases. The animals were divided into four series:

(1) Controls injected with normal saline solution;

(2) Controls treated with emulsion of ox hypophysis;

(3) Experimental animals treated with emulsion of tuber tissue of normal donors;

(4) Experimental series treated with tuber emulsion of hypophysis extract-treated donors.

Each animal received seven injections through the period of five days, being sacrificed on the fifth.

*Ox gland is triturated with sterile quartz sand and emulsified in phenolated water — 0.4 per cent.

From each animal were taken the ovaries, hypophysis, thyroid and adrenals which were examined histologically by the ordinary methods. In addition the uteri were secured.

Control Series (1). The vaginal opening was still closed in each. The ovaries together weighed from 0.019 to 0.020 grams in the various cases. Maturation was little advanced. Histological examination showed numerous primordial follicles with only a few in advanced stadia. Few or no atretic follicles were present (Fig. 1). The uterine horns showed development normal for the age (47 days).

Hypophysis Extract-Treated Controls (2). The vaginal orifice was open in each of the four animals. The ovaries weighed 0.037, 0.058, 0.049 and 0.027 grams, respectively, as compared with 0.020 grams in the normal

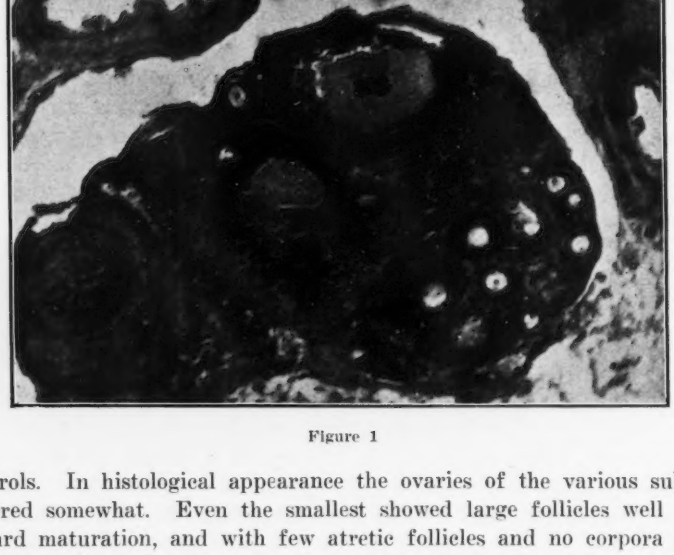

Figure 1

controls. In histological appearance the ovaries of the various subjects differed somewhat. Even the smallest showed large follicles well along toward maturation, and with few atretic follicles and no corpora lutea. The other three showed more advanced stadia of maturation consonant with their greater weights. All showed dilated vessels and mature follicles as well as some corpora lutea (Fig. 2). The uterine tubes were hypertrophic and showed marked development of villi in the internal lumina.

Rats Treated with Emulsion of Tuber Tissue from Normal Dogs (3). The changes were less pronounced than in the preceding case. In three instances the vulvar ostia were open. In these instances the ovaries weighed, respectively, 0.028, 0.030 and 0.027 as compared with 0.020 in normal controls. In the fourth case the vaginal orifice was still closed and the ovaries weighed only 0.021. Consonant with the weight, the histo-

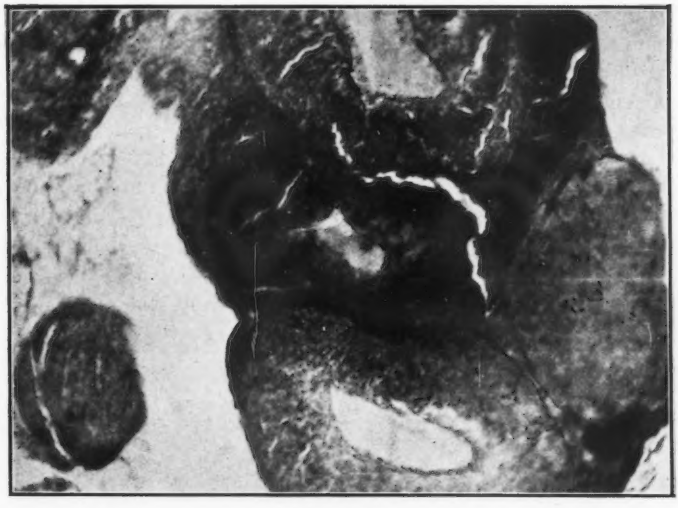

Figure 2

logical picture also showed more development than in the first series and less than in the second. The primordial follicles were absent, all being advanced somewhat toward maturation. Many atretic follicles were seen and the capillaries were full of blood. Figure 3 illustrates the findings.

Rats Injected with Tuber Emulsion from Dogs Treated with Hypophysis Extract (4). In this series also the vulvar orifice was open in three

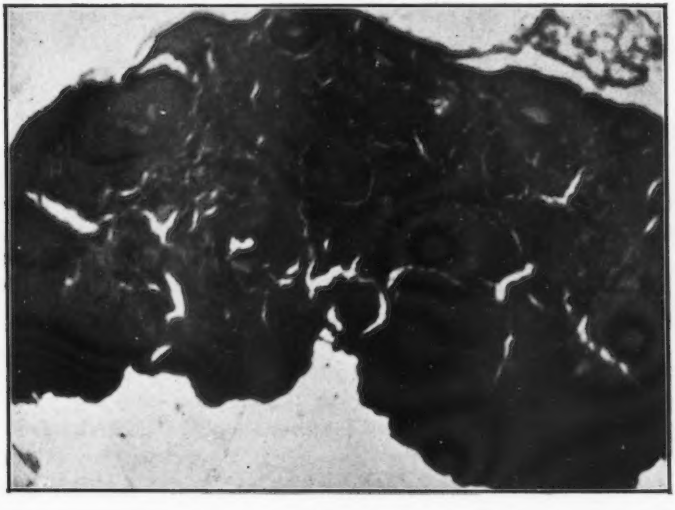

Figure 3

cases and closed in one. The ovaries weighed, respectively 0.030, 0.021, 0.029 and 0.020. The histological findings were very similar to those in the preceding series. That is, the ovaries were more advanced than in the normal controls and less advanced than in the extract-treated controls. Large and mature follicles were predominant. Few primordial follicles were seen (Fig. 4). The stroma was abundant and numerous atretic follicles were present. There were no corpora lutea. The uterine tubes were hypertrophic as before and showed marked development of the villi.

As regards the other endocrine glands the only significant changes from normal were turgidity of the follicular epithelium and rarefaction of the colloid of the thyroids of all injected animals and especially those treated with extract of the hypophysis; medullary hyperemia in the adrenals of all the injected animals.

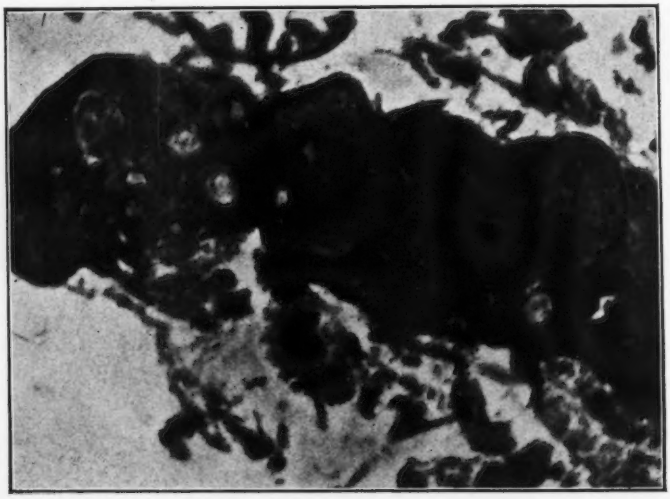

Figure 4

Not only does the tuber cinereum of man contain, as previously reported, a substance having a stimulating influence upon the sexual apparatus of rats but that of the dog shows a similar influence. The effect of the tuber extracts was qualitatively similar to, but quantitatively less extensive than, the effect of anterior hypophyseal extract. Only the latter carried development to the corpus luteum stage. It is possible that the findings indicate the presence of only prolan A in tuber extracts whereas both A and B are found in extract of the anterior pituitary. Possibly, however, had the experiments been continued longer, i.e., had more donor material been available, development might have been carried to the corpus luteum stage. Such effect was obtained in the previous research with human tuber tissue.

It appeared from these experiments that previous treatment of the donors with anterior pituitary extract did not enhance the hormone content of their tubera cinerea. That the pituitary extract used was genuinely potent is indicated by the comparative findings in the uninjected dogs and in those that received the extract. The hypophyses of the latter were richer in eosinophil cells. The epithelium of the thyroids was more turgid and the colloid more rarefied. Especially the ovaries contained some maturing graafian follicles while in the controls only primordial follicles were seen.

The presence of an active hormone substance at least similar to, if not identical with, the sex-stimulating principle of the anterior pituitary suggests that its action in part at least is on the cells of the hypothalamus. It probably represents hormone transported from the pituitary. It may be, however, that it is secreted in situ since Poppi (5), Scharer (6) and Roussy (7) have ascribed to the cells of this region a secretory function.

SUMMARY

Four groups of four young female rats each were injected, respectively, with (1) normal saline solution, (2) anterior pituitary emulsion, (3) emulsion made from the region of the tuber cinereum of normal dogs, and (4) an emulsion made from the same region of dogs treated with anterior pituitary extract. Results in the last two series were similar, showing a certain degree of maturation of the ovaries and hypertrophy of the uterine tubes but less in amount than was obtained from the pituitary extract.

REFERENCES

1. Pighini, G.: Riv. sper. di freniat. **56**: 575. 1932.
2. Collin, R. and J. Watrin: Compt. rend. Soc. de biol. **111**: 910. 1932.
3. Pighini, G.: Biochim. e terap. sper. **20**: 161. 1933.
4. Pighini, G. and L. Santoni: La nuova veterinaria. 1933.
5. Poppi: Riv. di pat. nerv. **36**: 397. 1930.
6. Scharrer, E.: Ztschr. f. vergl. Physiol. **17**: 491. 1932. Ztschr. f. d. ges. Neurol.
7. Roussy, G. and M. Mosinger: Compt. rend. Soc. de biol. **115**: 1143. 1934.

THE EFFECTS OF PITUITRIN, PITRESSIN AND PITOCIN UPON THE COPPER-REDUCING SUBSTANCES IN THE SERUM AND URINE OF DOGS

A. R. McINTYRE, R. F. SIEVERS AND H. F. ELIAS
From the Pharmacological Laboratory
University of Nebraska, Medical College
OMAHA

Borchardt (1), it appears, was the first to report glycosuria in animals as the result of the injection of posterior-lobe pituitary extracts of hypophysis. Working with dogs and rabbits, he described the onset of dextrosuria and its persistence in some cases for several days following the administration of horse pituitary extract. Subsequently an increasing number of papers have made their appearance on the subject of pituitary hyperglycemia and the glycosuria that commonly results from the injection of Liquor Pituitarii. Unfortunately the growth in the volume of data has not been concomitant with any unanimity of opinion as to the facts and still less as to their interpretation. Thus, while there is general agreement that hyperglycemia follows the injection of posterior lobe pituitary preparations (2) (3), this augmentation of blood sugar has been the subject of much discussion as to its immediate cause. Early workers, perhaps influenced to some extent by the investigations of Macleod (4) upon the effects of the stimulation of the splanchnics upon blood sugar, were inclined to attribute the hyperglycemia to a nervous mechanism acting upon a hypothetical center (5) (6). Later the hyperglycemia was attributed to a generalized action upon the sympathetic system (7). La Barre (8) believes the effect upon blood sugar to be indirect and mediated by adrenaline on one hand and by an action on the Islands of Langerhans on the other. Similarly Cowley (9) seeks an explanation for the hypoglycemic action of hypophysectomized dogs' blood in its possibly increased insulin content, thus tacitly implying the probability of a hormone antagonism. There seems to be small doubt of an interhormal control of carbohydrate metabolism. Dresel (10), Gigon (11), Achard, Ribot, Binet (12), Cammidge (13), Bowman and Grabfield (14), Goetsch, Cushing and Jacobson (15) and several investigators have sought modifications in the hepatic glycogen content following the administration of posterior-lobe pituitary preparations; for example, Bischoff and Long (16). Lambie (17) demonstrated that doses of pituitrin sufficient to prevent insulin hypoglycemia in anesthetized animals fail to do so when the liver is excluded and sufficient glucose is administered to maintain the initial glucose level during the control period. On the other hand, there has been failure to find loss of liver glycogen following the injection of presumably active extracts of hypophysis in fasting rats (18) and in rats on a normal diet (19). A rather recent paper suggests that the hepatic glycogen may be

augmented by pitressin (20). It would seem from a consideration of most of the available papers on this topic it is relatively certain that the hyperglycemia may be attributed in part at least to the mobilization of liver-glycogen and that while pituitrin prevents or decreases the fall in blood sugar produced by insulin it is unable to prevent the decrease in blood phosphates which commonly accompanies the insulin hypoglycemia (21) (22). As was pointed out by Voegtlin, Thompson and Dunn (23), much of the confusion seems to be attributable to the use of unstandardized material. In addition there appears to be failure on the part of some investigators to recognize that a portion of the copper-reducing substance in dogs' urine is non-fermentable by yeast. In a recent paper McIntyre and Sievers (24) described a decrease in the copper-reducing substance in dog urine that may accompany a simultaneous increase in the copper-reducing power of the serum subsequent to the injection of pituitrin.

As far as we are aware, this unlooked-for effect has not been described previously, hence a rather extensive series of experiments was performed

TABLE 1

DOG NO. 2. FEMALE, 15.1 KG.

Time	Specimen	Volume	Cu-reducing Substance Mg. 100 cc.*			Percentage Difference in Cu-reducing Substance		
			Total	Non-fermentable	Fermentable	Total	Non-fermentable	Fermentable†
8:40	Serum$_1$	..	143	..	..	..	..	..
8:55	Urine$_1$	15	52	48	4	..	..	..
9:03		15 units Liquor Pituitarii injected subcutaneously.						
9:35	Urine$_2$	23	51	47	4	− 2	− 2	0
10:02	Serum$_3$	..	170	..	..	19	..	..
10:05	Urine$_3$	28	42	34	8	−19	−29	100
11:04	Urine$_4$	25	45	40	5	−13	−20	25
11:55	Urine$_5$	15	55	50	5	6	4	25
2:50	Serum$_3$	..	173	..	..	21	..	..

*Average of two determinations by the Benedict modification of the Folin-Wu method and expressed as Mg. of dextrose.

†See the remarks in the discussion as regards the percentage changes in the fermentable substances.

in the hope of discovering which of the fractions of posterior-lobe extract was responsible and under what conditions this decrease in copper-reducing substance in the urine occurred. Accordingly the following experiments were performed on a group of 8 dogs, 2 male and 6 female:

1. The effects of pituitrin upon the copper-reducing substance in the serum and its effects upon the fermentable and non-fermentable copper-reducing substances in the urine (12 experiments).

2. Similar experiments with the alpha fraction of posterior-lobe extract (Pitocin)* (10 experiments).

3. Similar experiments with the beta fraction of posterior-lobe extract (Pitressin) (12 experiments).

*Adequate supplies of Pitocin and Pitressin were kindly presented by Doctor Oliver Kamm of the Parke Davis Laboratory. The pitocin was stated to contain 0.2 units of the pressor substance in 10 units of the oxytocic material and the pitressin 0.3 oxytocic units in 10 units of the pressor material.

4. Experiments in which both the alpha and beta fractions were used together (3 experiments).

TECHNIQUE

The animals were maintained on an adequate diet and were in a healthy condition throughout the experimental period. Samples of blood were collected and prepared for the determination of blood sugar in duplicate in the manner previously described by McIntyre and Sievers (24). Urine was obtained by catheterization.† It was necessary to train the animals to remain quiet throughout the experiments as obviously any excitement would be apt to invalidate the results. After the collection of the initial samples of blood and urine 10 units of the material used was injected subcutaneously; this amount was equivalent to 0.47-0.83 units per kilogram of animal. In some of the early experiments somewhat larger amounts, approximately 1 unit per kilogram, were used. Subsequent samples of urine were usually collected 20 minutes, 1, 2, 3, and 5 hours

TABLE 2

DOG NO. 1. FEMALE, 19.4 KG.

Time	Specimen	Volume	Cu-reducing Substance Mg. 100 cc.*			Percentage Difference in Cu-reducing Substance		
			Total	Non-fermentable	Fermentable	Total	Non-fermentable	Fermentable†
10:10	Serum$_1$	..	137	..	..	..	..	..
10:15	Urine$_1$	42	59	50	9	..	..	..
10:17		20 units Liquor Pituitarii injected subcutaneously.						
10:40	Urine$_2$	11	57	50	7	3	0	−22
11:15	Serum$_2$	..	152	..	..	11	..	..
11:20	Urine$_3$	25	62	57	5	5	14	−45
11:50	Urine$_4$	14	64	51	13	8	2	45
1:40	Urine$_5$	29	63	51	12	7	2	33
2:15	Serum$_3$	..	161	..	..	18	..	..

*See the remark at foot of Table 1. †See the remark at foot of Table 1.

after the injection. However, it was not always feasible to adhere exactly to these times because of the difficulty in obtaining sufficient urine for analysis. Following the injection, samples of blood were generally collected at the end of the first hour and again after the last sample of urine was obtained. The urine was diluted and prepared for the determination in duplicate of the fermentable and non-fermentable copper-reducing substances by the method of Harding and Selby (25), to whom the reader is referred for an adequate discussion. In our hands the importance of thoroughly washing the commercial yeast was frequently demonstrated and neglect of this point was the chief cause of failure in our determinations. Satisfactory dilutions for dogs' urine are 1:5-1:10. The length of time during which fermentation should proceed has been the subject of discus-

†Females are easily catheterized when placed on their backs and held by an assistant. A small human rectal speculum is inserted into the vagina and then gently dilated, the external urethral orifice is thus rendered plainly visible and a human female glass catheter can be inserted and the animal turned on her side. Females of 10 Kg. weight or more that have not littered can be catheterized with equal facility. Males are best catheterized by means of a human urethral catheter.

sion; the question has been considered by Hiller, Linder and Van Slyke (26) and by Folin and Svedberg (27). Similarly, the question as to the true value for blood sugar as examined, for example, by Benedict (28), need not concern us here since no claim is made that the values presented are absolute and since our findings would not be materially influenced should the true values be somewhat lower or higher than our data indicate.

EXPERIMENTAL RESULTS

Four protocols are selected for presentation, two from experiments with pituitrin and two from experiments in which both pitressin and pitocin were injected. These protocols are selected because they illustrate rather clearly the opposite effects obtainable upon the copper-reducing substance of the urine as a consequence of the injection of posterior-lobe pituitary extracts. To conserve space protocols from the experiments in which pitocin and pitressin were used singly are not submitted as these substances produce changes in the serum and urine qualitatively similar

TABLE 3
DOG NO. 3. FEMALE, 14.3 KG.

Time	Specimen	Volume	Cu-reducing Substance Mg. 100 cc.*			Percentage Difference in Cu-reducing Substance		
			Total	Non-fermentable	Fermentable	Total	Non-fermentable	Fermentable†
9:20	$Serum_1$	..	136	..	..	..	..	..
9:25	$Urine_1$	42	42	38	4	..	..	..
9:31		5 units of pitocin and 5 units of pitressin injected subcutaneously.						
9:56	$Urine_2$	8	45	41	4	7	8	0
10:29	$Urine_3$	5	32	29	3	−24	−24	−25
10:31	$Serum_2$	..	162	..	..	19	..	..
11:31	$Urine_4$	6	43	38	5	2	0	25
1:06	$Urine_5$	5	51	47	4	21	24	0
3:20	$Serum_3$	..	180	..	..	32	..	..

*Average of two determinations by the Benedict modification of the Folin-Wu method and expressed as Mg. dextrose.

†See the remarks in the discussion as regards the percentage changes in the fermentable substances.

to those exhibited in the tables presented. The degree of hyperglycemia obtained with pitressin alone was not usually greatly different from that produced by pituitrin and in 12 experiments performed with pitressin a decrease in the urine copper-reducing substance was observed four times, similarly in 12 experiments with pituitrin a decrease in the urine of copper-reducing substance was also observed four times. With pitocin the increase in blood sugar seemed in general to be somewhat greater than with pitressin and a decrease in the urine copper-reducing substance was found only once in 10 experiments. In the three experiments performed in which pitressin and pitocin were injected simultaneously there was a decrease observed in the urine copper-reducing substance once. In all experiments with these extracts, whether used separately or together, there was always produced an increase in the blood sugar. Frequently the blood sugar level remained higher than its initial level after the urine copper-reducing substances had commenced to decline toward their original values. Often an

initial decrease in urine copper-reducing substance was followed by augmentation and after some hours an increase in the urine copper-reducing substances gave way to a progressive decrease, the final level becoming lower than that found during the control period. It will be seen that the effects on the fermentable substances in the urine are more transient than those on the blood sugar and frequently the fermentable substances in the urine commenced to decrease in amount while the serum glucose remained elevated.

DISCUSSION

An examination of these results reveals that changes in the concentration of the copper-reducing substances in the urine, brought about as a result of the injection of the above pituitary preparations in their present state of purity, can occur entirely independently of changes in the serum sugar; for while the latter is always increased, the copper-reducing substances in the urine are frequently decreased following the administration of posterior-lobe fractions. Neither does it appear that there is any parallel

TABLE 4
DOG NO. 1. FEMALE, 21.4 KG.

Time	Specimen	Volume	Cu-reducing Substance Mg. 100 cc.*			Percentage Difference in Cu-reducing Substance		
			Total	Non-fermentable	Fermentable	Total	Non-fermentable	Fermentable†
9:30	Serum$_1$	..	143	..	..	..	..	..
9:45	Urine$_1$	23	39	36	3	..	..	..
9:55		5 units of pitocin and 5 units of pitressin injected subcutaneously.						
10:15	Urine$_2$	12	48	42	6	23	17	100
10:50	Urine$_3$	14	53	47	6	36	30	100
11:04	Serum$_2$	..	167	..	..	17	..	..

*See the remark at foot of Table 3. †See the remark at foo of Table 3.

effect upon the non-fermentable and the fermentable copper-reducing substances in the urine. However, the apparently much large percentage increments and decrements in the concentration of the fermentable substances must be viewed with caution as the experimental error in the case of these small amounts of fermentable material will be reflected in a much larger percentage change than in the larger non-fermentable portion. It would seem that pitocin has a somewhat greater effect on the blood sugar than pitressin and that a decrease in the urine copper-reducing substance is much less likely to occur with the alpha than with the beta fraction. The diuresis commonly observed after the injection of pituitrin or pitressin does not appear to afford an entirely adequate explanation for the decrease in the concentration of the copper-reducing substances as in several instances the fermentable substances appear to increase while those that do not ferment decrease. It is difficult to imagine a mechanism whereby an abundance of glomerular filtrate could result in the simultaneous dilution of one of two presumably similar compounds and in the concentration of the other. It seems more probable that perhaps some local change in the

selective activity of the renal epithelium is brought about as a result of the action of these preparations. Burgess, Harvey and Marshall (29) have recently presented good indirect evidence that a localized action on the renal tubule is exerted by pitressin resulting in an increase of the rate of water resorption from the loop of Henle. It seems rather significant that in our experiments pitocin, which had a negligible diuretic effect, caused a decrease in the urine copper-reducing substances only once in 10 experiments.

SUMMARY AND CONCLUSIONS

An investigation has been made of the changes brought about in the concentrations of the copper-reducing substances in the serum and the fermentable and non-fermentable copper-reducing substances in the urine in 8 dogs as a result of their injection with pituitrin, and pitocin and pitressin separately and together. The results of our experiments are summarized below:

1. In dogs that have fasted 18 hours the copper-reducing power of the serum as measured by the Benedict modification of the Folin-Wu technique is increased following the subcutaneous injection of pituitrin, pitressin or pitocin.

2. The copper-reducing substances in the urine are sometimes decreased or they may be increased following such injections, and frequently an initial decrease is followed by an increase and an increase may be followed after some hours by a decrease to below the initial concentration.

3. Changes in the urine copper-reducing substances do not appear to bear any relationship to such changes in the serum.

4. There does not appear to be any proportionality between the fluctuations in concentration of the fermentable and non-fermentable urine copper-reducing substances.

REFERENCES

1. Borchardt, L.: Ztschr. f. klin. Med. **66**: 332. 1908.
2. Stenstrom, Th.: Biochem. Ztschr. **58**: 472. 1914.
3. Lawrence, R. D. and R. F. L. Hewlett: Brit. M. J. (1): 998. 1925.
4. Macleod, J. J. R.: Am. J. Physiol. **21**: 373. 1908.
5. Weed, L. H., H. Cushing and C. J. Jacobson: Bull. Johns Hopkins Hosp., **24**: 40. 1913.
6. Keeton, R. W. and F. C. Becht: Am. J. Physiol. **49**: 248. 1919.
7. Fritz, G.: Arch. f. d. ges. Physiol. **220**: 101. 1928.
8. La Barre, J.: Arch. internat. de pharmacodyn. et de thérap. **38**: 409. 1930.
9. Cowley, R. J.: J. Pharmacol. & Exper. Therap. **43**: 287. 1931.
10. Dresel, K.: Ztschr. f. exper. Path. u. Therap. **16**: 365. 1914.
11. Gigon, A.: Biochem. Ztschr. **174**: 257. 1926.
12. Achard, Ch., A. Ribot and L. Binet: Compt. rend. Soc. de biol. **82**: 788. 1919.
13. Cammidge, P. U.: Proc. Roy. Soc. Med. **19**: 37. 1926.
14. Bowman, K. M. and G. P. Grabfield: Endocrinology, **10**: 201. 1926.
15. Goetsch, E., H. Cushing and C. J. Jacobson: Bull. Johns Hopkins Hosp. **22**: 165. 1911.
16. Bischoff, F. and M. L. Long: Am. J. Physiol. **97**: 215. 1931.
17. Lambie, C. G.: Brit. J. Exper. Path. **7**: 22. 1926.
18. Lawrence, R. D. and R. A. McCance: Biochem. J. **25**: 570. 1931.
19. Fukui, T.: Arch. f. d. ges. Physiol. **210**: 427. 1925.

20. Bischoff, F. and M. L. Long: Am. J. Physiol. **99**: 253. 1931.
21. Lambie, C. G. and F. A. Redhead: Biochem. J. **23**: 608. 1929.
22. Geiling, E. M. K., A. De Lawder and M. Rosenfeld: J. Pharmacol & Exper. Therap. **42**: 263. 1931.
23. Voegtlin, C., J. W. Thompson and E. R. Dunn: J. Pharmacol. & Exper. Therap. **25**: 137. 1925.
24. McIntyre, A. R. and R. F. Sievers: J. Pharmacol. & Exper. Therap. **49**: 229. 1933.
25. Harding, V. J. and D. L. Selby: Biochem. J. **25**: 1815. 1931.
26. Hiller, A., G. C. Linder and D. D. Van Slyke: J. Biol. Chem. **64**: 625. 1925.
27. Folin, O. and A. Svedberg: J. Biol. Chem. **70**: 405. 1926.
28. Benedict, S. R.: J. Biol. Chem. **68**: 759. 1926.
29. Burgess, W. W., A. M. Harvey and E. K. Marshall, Jr.: J. Pharmacol. & Exper. Therap. **49**: 237. 1933.

EXPERIMENTS WITH THEELIN AND GALACTIN ON GROWTH AND FUNCTION OF THE MAMMARY GLANDS OF THE MONKEY

EDGAR ALLEN, W. U. GARDNER* AND A. W. DIDDLE
Department of Anatomy, Yale University†
NEW HAVEN

The present investigation of the galactopoietic hormone of the pituitary in the monkey was undertaken for the purpose of determining optimum conditions for mammary response, the possible secondary or accompanying effects of the hormone, and the effect of theelin on conditioning the mammary glands for response to galactin.

During the past five years numerous investigators have observed the galactopoietic function of certain pituitary extracts. It was concluded by Gardner and Turner (1933), and more extensively demonstrated by Riddle, Bates, and Dykshorn (1933), that the lactation-stimulating material, designated as galactin and prolactin, respectively, by these investigators, was distinct from the gonad-stimulating or growth-stimulating hormones. Riddle, Bates, and Dykshorn (1933a) also distinguished it from the thyrotropic material of the pituitary.

The lactation-stimulating hormone has been found to be capable of stimulating the secretory activity of the mammary glands of various rodents, carnivora, ungulates, and of at least one primate. In conjunction with C. G. Hartman, Riddle, Bates, and Dykshorn (1933) injected three adult monkeys with prolactin. One monkey was ovariectomized and hysterectomized on the twelfth day of the menstrual cycle, a second was hysterectomized on the eleventh day, and the third was normal. None of the animals were lactating at the time injections were started. Milk secretion was induced within six days after the treatment began.

Advances in purification and concentration of hormone extracts increase the possibility of clinical applications. Species variations in the effects of the sex- and gonad-stimulating hormones warn against too intensive generalization, though broadly speaking, hormone effects are not species specific. Not only should the possibility of species variations be considered in extending laboratory observations to clinical application, but the possibility of accompanying harmful functional disturbances through damage to associated endocrines should be thoroughly investigated. In addition to species variations in response to hormones, individual variations exist which are determined by the developmental and functional states of the organs or tissues on which the hormones act. For example, for the stimulation of extensive lactation, the mammary gland

*National Research Council Fellow.

†This work was supported by a grant to the senior author from the Committee for Research in Problems of Sex of the National Research Council.

must be morphologically complete or nearly so. For response to corporin or progestin, the uterus and the mammary glands must first be developed to the oestrous condition by the action of the ovarian follicular hormone.

MATERIALS AND METHODS

Ten Macacus rhesus monkeys, weighing from 3365 to 6050 grams and roughly representing three different age groups, were used. Four of the animals were sexually mature, four were becoming sexually mature as was judged by the onset of menstruation, and the reddening and swelling of the sexual skin, and two were sexually immature. Doses ranging from 3 to 13 rabbit units of galactin or prolactin were injected subcutaneously for periods varying from 7 to 13 days. Two injections were given each day. Normal animals at various times of the menstrual cycle and ovariectomized and partially ovariectomized animals were treated following various preliminary treatment with ovarian follicular hormone (theelin* or amniotin†). Protocols given below give further details relating to the animals and the treatment.

A part of the lactation-stimulating hormone used was prepared in the laboratory and a part of it was "prolactin."† To differentiate between the two, galactin will be used to designate the former. The galactin was prepared from fresh porcine pituitaries. All extracts were assayed for potency in either rabbits or pigeons or both. The monkeys were injected subcutaneously in the leg or thigh regions.

Seven of the monkeys were autopsied immediately after the injections were stopped (RH, C, RC, LHY, R, LBR, and RB). Gross and histological examinations were made of the following tissues in addition to the mammary glands: thyroid, parathyroid, pituitary, suprarenal, uterine tubes, uterus, and vagina. Sections of mammary gland were removed at biopsy from three animals (BR, HR, and YH).

PROTOCOLS OF EXPERIMENTS

Mature Monkeys

Monkey BR, weight 5700 grams. At laparotomy performed on the twentieth day of the menstrual cycle (27-28 day type) a recent corpus luteum and a large follicle were found in the right ovary. No milk could be expressed from the mammary glands. Control sections of mammary tissue were not removed at this time. Over nine rabbit units of prolactin were injected during the next nine days. A milky fluid was removed from the glands on the sixth day. A second gland was removed on the tenth day. Histological preparations revealed a functional mammary epithelium. Lactation continued in the other gland for four days following cessation of treatment. Menstruation began on the last day of treatment at the normal time.

Monkey HR, weight 6050 grams. A recent corpus luteum was found at a laparotomy performed on the fifteenth day of a menstrual cycle of the 28-day type. A section of the right mammary gland was also removed. Gross and microscopic preparations gave no indication of secretory activity (Fig. 1). No milk or fluid could be removed from either gland. Galactin treatment was begun immediately and nearly nine rabbit units were injected in nine days. Milk was expressed from the left gland on the seventh day of treatment. A second sec-

*We wish to express our appreciation to Parke, Davis Company and to Dr. Oliver Kamm for the supplies of theelin used in this experiment.

†The prolactin and amniotin was generously supplied by E. R. Squibb & Company through Dr. J. H. Anderson, to whom we express our appreciation.

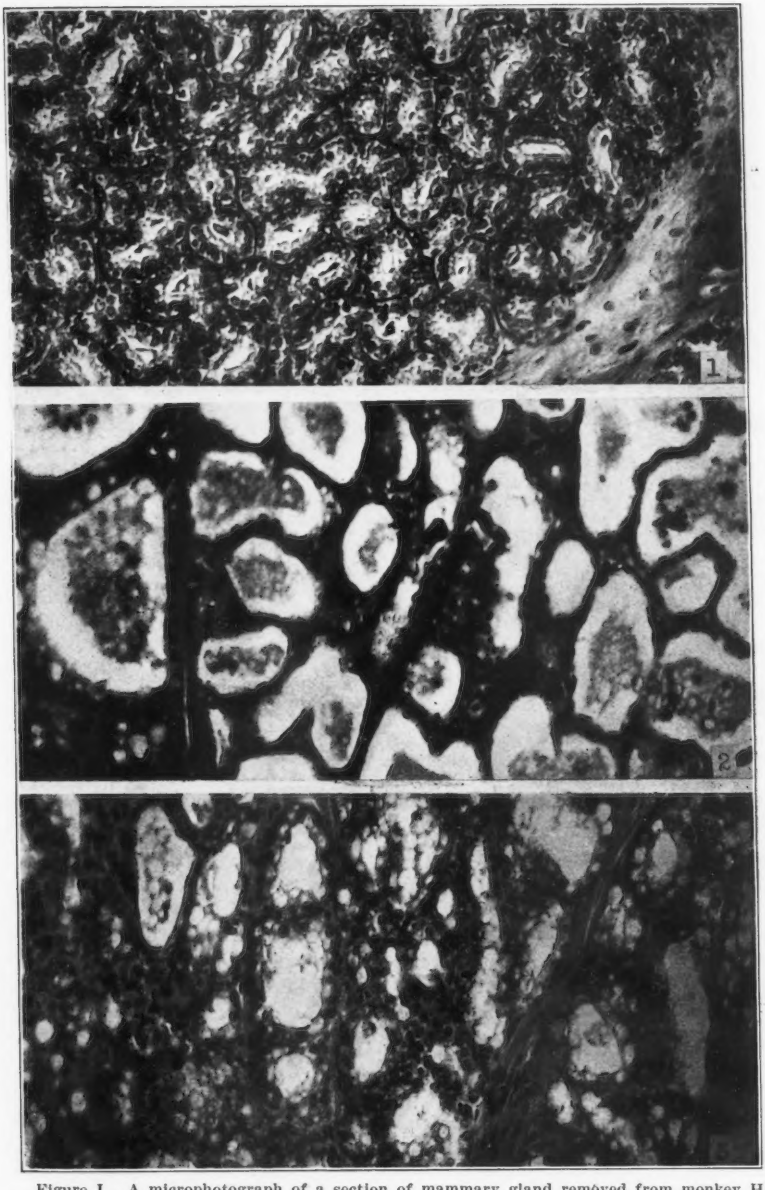

Figure I. A microphotograph of a section of mammary gland removed from monkey HR on the 15th day of a menstrual cycle and immediately before injections of galactin were started. No milk or fluid could be obtained from the glands. The alveoli were small. Broad connective tissue septa separated the lobules. 160 x.

Figure 2. A microphotograph of a section of mammary gland removed from monkey HR 10 days after the gland shown in Figure 1. Nearly 9 rabbit units of galactin had been injected in the preceeding days. The alveoli were distended with the contained secretion. The epithelium was vascuolated as in actively secreting glands. Milk was obtained from the nipples after the seventh day of treatment. 160 x.

Figure 3. A microphotograph of a section of mammary gland of monkey RC removed after 1700 rat units of theelin and over 6 rabbit units of galactin had been subcutaneously injected during ten and nine days respectively. The alveoli were large and filled with secretion. The epithelial cells contained numerous secretion vacuoles. 160 x.

tion of the right gland was removed on the tenth day. The alveoli of some of the lobules were distended with secretion (Fig. 2). In other lobules a vacuolated epithelium indicated secretory activity.

Monkey YH, weight 4865 grams. One ovary was removed before the experiment was started. A section of the right mammary gland was removed before injecting prolactin. The lobules were well developed but not distended. Slightly over six rabbit units of prolactin were injected during nine and one-half days, beginning on the twenty-eighth day of the menstrual cycle which had been previously irregular. A watery fluid was expressed from both breasts at the time injections were begun. Milk was expressed from the nipples on the fifth day of treatment. A second section of gland removed at biopsy on the tenth day revealed an active mammary epithelium and a small accumulation of milk in the alveoli and ducts.

Monkey RH, weight 4860 grams. Beginning twelve days after ovariectomy 2150 rat units of amniotin were first injected over an interval of eleven and one-half days. Injections of prolactin were begun immediately following the amniotin treatment, slightly over six rabbit units being given in nine and one-half days. As this monkey had been recently suckling young a few drops of milk were obtained from the nipples at the time theelin injections were begun. A section of the right mammary gland was removed before prolactin injections were started. There was no histological evidence of secretory activity. On the seventh day of prolactin treatment 1 to 1½ cc. of milk were expressed. A second gland was obtained at autopsy on the tenth day. The gland was but slightly thickened. The alveoli were only slightly distended.

Monkeys Just Reaching Sexual Maturity

Monkey C, weight 4750 grams. Ovariectomy was performed on the second day of the menstrual cycle. A section of mammary gland was also removed. Though lobules were present the alveoli were very small. Eleven hundred rat units of theelin were injected over an interval of eleven days. A second section of gland removed following the theelin injections showed little change. Three rabbit units of galactin were then given during the next seven days. Two hundred rat units of theelin were also injected during the first four days of galactin treatment. A small amount of watery fluid was squeezed from the glands on the last day of injections. A third section of mammary gland obtained at autopsy on the eighth day showed no evidence of secretory activity.

Monkey RC, weight 4200 grams. Monkey RC was taken on the third day of the menstrual cycle and treated in the same manner as animal C, except that 1700 rat units of theelin and over six rabbit units of galactin were given during ten and nine days, respectively. A small amount of milk was recovered from the nipples at the time of autopsy. The gland removed was thickened and the alveoli were distended with milk. Histological examination showed an actively functioning gland (Fig. 3).

Monkeys LHY and R, weight 3750 and 3650 grams, respectively. The left ovary of monkey LHY was removed previous to theelin injections for histological study. A laparotomy was performed on monkey R. Neither corpora lutea nor large follicles were present in the ovaries of either animal. Animal R had menstruated twice within the past two months. Monkey LHY had never menstruated. A total of 1700 rat units of theelin were injected into each animal over a period of ten days. An extract of galactin, *from which the gonad-stimulating hormone had not been removed*, was substituted for theelin on the eleventh day of treatment and was continued for ten days. A total of seven and one-third and eight rabbit units were injected into monkeys LHY and R, respectively. Examination of the ovaries before treatment showed no corpora lutea or large follicles present. Each ovary after treatment contained several large follicles, two or three of which were hemorrhagic. The right ovary of animal LHY measured 18x16x14 mm., or an increase of 8x6x2 mm. over the control measurement. The right and left ovaries of monkey R measured 16x15x13 mm. and 18x15x13 mm., or 8x6.5x6 mm. and 9x8x7 mm. more than the measurements taken at laparotomy, respectively. Mammary glands were removed both before and after galactin injections. The glands of LHY, though large (measuring 62x65 mm.) showed but partial lobule development. No alveoli were present in glands of monkey R though the glands measured 67x45 millimeters (Figs. 6 and 7).

Monkeys LHR and RB, weight 3365 and 3630 grams, respectively. Animal RB had menstruated once previous to this experiment. Monkey LHR had not passed through the first menstrual period. Each animal received 1700 rat units

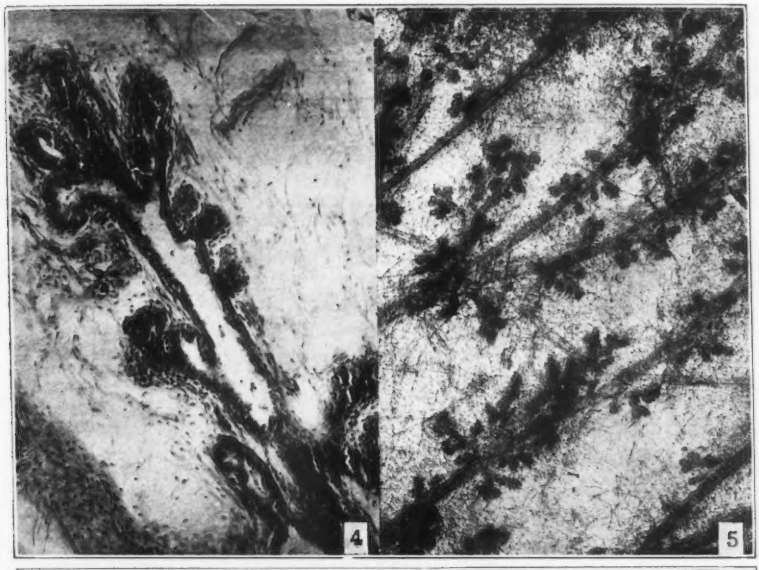

Figure 4. A microphotograph of a mammary gland duct and its side branches from monkey R. The terminal branches are probably interlobular ducts. Seventeen hundred rat units of theelin and eight rabbit units of galactin had been injected during two respective 10-day periods. Sections of glands removed prior to treatment were comparable. The gland removed at autopsy measured 67x45 mm. 85 x.

Figure 5. A section of whole gland preparation of monkey R removed at the same time as the gland shown in Figure 6. The gross structure of the gland closely resembles that of the smaller gland in Figure 5. Monkey R had menstruated twice. 16 x.

Figure 6. A microphotograph of a section of mammary gland of monkey LHR (3365 gms.) removed after 1700 rat units of theelin and nine rabbit units of galactin had been injected during ten and thirteen days respectively. No alveoli were present. Secretory activity was not induced. Figure 5 is a whole gland preparation. The gland measured grossly 33x24 mm. 85 x.

Figure 7. A section of a whole gland preparation of monkey LHR. Though the glands were small, the smaller ducts were extensively developed. The smaller terminal ducts are probably interlobular ducts. 16 x.

of theelin in ten days. This was stopped abruptly and galactin substituted on the eleventh day. Galactin injections were continued for thirteen days (the same extract was used as for monkeys LHY and R). A total of thirteen and one-half rabbit units were injected into monkeys RB and LHR, respectively. Over three rabbit units of the amount given monkey RB were administered during the last two days of treatment. One and one-half units were injected intravenously fourteen hours before the animal was killed in order to observe the effects of large amounts of the gonad-stimulating hormone on the ovaries. No fluid could be expressed from the mammary glands of either animal. The glands of monkey LHR were small and but partially developed (measured 26x18 mm. and 33x24 mm.) (Figs. 4 and 5). Though the mammary glands of animal RB were much larger (76x59 mm.) no lobules were present.

DISCUSSION

According to the age-weight relationship published by Hartman and Straus (1933) the younger monkeys used were about three years of age, the older individuals being presumably between five and six years of age. All of the monkeys except LHR and LHY had menstruated at least once and as judged from the absence of corpora lutea ovulation had occurred in only four of the larger animals. The sexual skin of monkeys LHR and LHY had been undergoing periodic swelling.

The five larger animals gave definite evidence of the initiation of mammary secretory activity. In three animals this was particularly pronounced. In no case was the degree of hypertrophy of the mammary glands equal to that observed in other experimental animals such as the rabbit or the dog in which the glands become tremendously thickened with the accumulated milk. As but one observation has been made on the mammary glands of a parturient monkey (Turner and Allen, unpublished) and none have been made on the glands of normally lactating monkeys, a comparison of the experimentally induced mammary hypertrophy with that normally occurring cannot be made.

Additional interesting observations were made on the normal development of the mammary glands of the Macacus rhesus. The duct system alone develops initially. The mammary glands of monkeys RB and LHY, though large in total extent, had developed only a duct system. In the former small branches, probably intralobular ducts, were present. In the latter small alveolar buds had developed from the small intralobular ducts. These observations are in full agreement with those of Turner and Allen (1933), who stated, "The prepubertal stages show varying stages of duct development." The mammary glands of the normally maturing monkeys reach a large size but exist only as duct systems. From the specimens studied to date it seems that the further development of the finer structures of the mammary glands occurs either late in the prepubertal period or after the appearance of puberty.

Allen (1927) and Turner and Allen (1933) observed a growth of the ducts and recovery from castrate atrophy in the mammary glands of monkeys following injections of theelin. There was little or no evidence from the present experiments that theelin greatly stimulated the further development of the glands during the short time that it was injected. The

mammary glands of the monkey respond much more slowly than those of the rabbit and the mouse. From the control glands removed at various times in the menstrual cycle in the different animals no evidence for extensive cyclic hyperplasia or regression of the glands was obtained.

As with the rabbit, guinea-pig, and rat (Nelson and Pfiffner, 1931; Gardner and Turner, 1933, and Riddle, Bates, and Dykshorn, 1933) extensive lactation was induced only in the fully developed mammary glands. Unlike the rabbit (Gardner and Turner, 1933), a partial lactation in the monkey was not produced when galactin was injected following a previous theelin treatment of animals with but partially developed mammary glands.

SUMMARIZATION OF THE TREATMENT AND RESULTS OBTAINED IN TEN MACACUS RHESUS MONKEYS

Monkey	Weight in Grams	Sexual Maturity	Castrate or Normal	Theelin Injected		Condition of Mammary Glands		Treatment Galactin or Prolactin	
				Days	Units	Before Galactin	After Galactin	Units	Days
BR	5700	Mature	Normal, 20th day of cycle	..	....	Mature	Lactation	9	9
HR	6050	Mature	Normal, 15th day of cycle	..	....	Mature	Lactation	9	9
YH	4865	Mature	Semiovariectomy	..	....	Mature	Lactation	6	9½
RH	4860	Mature	Total ovariectomy	11½	2150	Mature	Lactation (slight)	6	9½
C	4200	Early maturity	Total ovariectomy	13	1300	Almost mature	No effect	3	7
RC	4750	Early maturity	Total ovariectomy	11	1700	Mature	Lactation	6	9
HY	3750	Early maturity	Semiovariectomy	10	1700	(62x65 mm.) partial lobule development	No effect	7½	9
R	3630	Early maturity	Normal	10	1700	(67x45 mm.) No lobules	No effect	8	9
LBR	3365	Immature	Normal	10	1700	(26x18 and 33x24 mm.) No lobules	No effect	9½	12½
RB	3630	Immature	Normal	10	1700	(26x59 mm.) No lobules	No effect	13	13

In the rabbit galactin will induce a limited secretion in the partially developed glands if such glands have been recently in the presence of theelin, either that produced by the animal's own ovaries or injected (Gardner and Turner, 1933). Sufficient secretory epithelium is not present, however, to produce the great hypertrophy of the glands observed during normal lactation or the even greater hypertrophy observed following galactin treatment.

From the descriptions given in the protocols and from the results tabulated in the accompanying table, it seems that complete sexual maturity and complete morphological development of the mammary glands are

necessary to obtain a positive galactin or prolactin reaction, mature animals responding positively and immature animals negatively. Nelson (1934) has shown that in the guinea-pig theelin is indirectly antagonistic to the lactation-stimulating principle though it prepares the glands for lactation. In this manner he explains the mechanism for the control of normal lactation.

Though the observations on the genital tract and other glands may have been complicated by the previous theelin treatment or castration, it was concluded that galactin or prolactin had no apparent effect on the other tissues or glands observed. The expected reddening and swelling of the "sexual skin" was present in all of the animals treated with theelin. Enlargement of the cervix and increased activity of the cervical glands were observed uniformly. Extravasation of blood beneath the uterine mucosa was observed microscopically at autopsy in the uterus of monkey RC. This animal was treated for seven days with galactin after theelin treatment was abruptly discontinued. Such a condition of bleeding has been described after theelin treatment (Allen, 1927; 1928). Monkey C failed to show the submucosal bleeding as the theelin dosage was gradually reduced during the first four of the seven days of galactin treatment.

In the four younger monkeys consideration must be made of the effect of the gonad-stimulating hormone contained in the galactin extract. Although injections of theelin were abruptly discontinued before galactin treatment was begun, it is probable, since reddening and swelling of the sexual skin increased during galactin treatment, that the theelin was again restored to a relatively high level as large follicles were produced in the ovaries. Ordinarily rapid regression of these characteristics occur with cessation of injections of theelin in animals at this stage of maturity (Allen, 1932). Theelin was supplied by the ovaries of monkeys HR and BR. The two latter were followed for three months after the experiment with galactin. The menstrual cycles remained regular in duration and in amount of flow. Ovaries of monkeys RH, C, and RC sectioned following galactin or prolactin injections contained normal follicles in all stages of development comparable to those of an untreated animal. Ovaries of the four animals which received extracts containing the gonad-stimulating hormone, in addition to the lactation-stimulating hormone, contained predominately large follicles. In each ovary two to three hemorrhagic follicles were found on gross examination.

As far as could be determined grossly and microscopically, none of the other endocrine glands examined gave any indication of change following the administration of the lactation-stimulating hormone extracts. Riddle and Bates (1933) reported a decrease in the size of the testis of the male dove following the activation of the crop glands. Similar observations have been made in our laboratory. From the results of investigations performed up to this time, the inhibition decrease of testis weight in the pigeon appears to be the only inhibiting effect of the hormone and this effect may be indirect.

SUMMARY

1. Ten Macacus rhesus monkeys weighing from 3365 to 6050 grams and at various stages of sexual maturity were injected with galactin, or prolactin, with or without previous theelin treatment.

2. Lactation was induced in four mature animals and in one animal just reaching sexual maturity, following the injections of six to nine rabbit units of galactin or prolactin. Three of the mature animals possessed one or both ovaries. The other two were ovariectomized. The two ovariectomized animals had been previously injected with 2150 and 1700 rat units of theelin. Treatment with galactin was begun in three of the above normal animals on the third, fifteenth, and twentieth days of the menstrual cycle, respectively.

3. The lactation-stimulating hormone in doses from three to thirteen rabbit units did not induce lactation in the remaining five animals, three of which were just reaching maturity and two of which were immature. One animal was ovariectomized. From 1300 to 1700 rat units of theelin were injected in all cases before beginning galactin or prolactin treatment.

4. All of the monkeys with mature mammary glands responded positively to galactin or prolactin in the doses and over the interval given. Partially developed mammary glands of younger monkeys did not respond.

5. No histological changes were observed in the reproductive tract, thyroid, parathyroid, pituitary or suprarenal glands that might be attributed to the injections of the lactation-stimulating hormone.

REFERENCES

Allen, E.: The menstrual cycle of the monkey, Macacus rhesus: Observations on normal animals, effects of the removal of the ovaries and the effects of injections of ovarian and placental extracts into spayed animals. Contrib. Embryol. (No. 98), Carnegie Inst., Wash. **19**: 1. 1927.

Allen, E.: Further experiments with an ovarian hormone in the ovariectomized adult monkey, Macacus rhesus, especially during the degeneration phase of the experimental menstrual cycle. Am. J. Anat. **42**: 467. 1928.

Allen, E.: Ovarian follicular hormone, theelin; animal reactions. Chapter IX, Sex and Internal Secretions, Williams and Wilkins Co., Baltimore. 1932.

Gardner, W. U. and C. W. Turner: The function, assay, and preparation of galactin, a lactation stimulating hormone of the anterior pituitary and an investigation of the factors responsible for the control of normal lactation. Mo. Agr. Exper. Sta. Res. Bull, 196. 1933.

Nelson, W. O.: Studies on the physiology of lactation. III. The reciprocal hypophyseal ovarian relationship as a factor in the control of lactation. Endocrinology, **18**: 33. 1934.

Nelson, W. O. and J. J. Pfiffner: Studies on the physiology of lactation. I. The relation of lactation to the ovarian and hypophyseal hormones. Anat. Rec. **51**: 51. 1931.

Riddle, O., R. W. Bates and S. W. Dykshorn: The preparation, identification, and assay of prolactin—a hormone of the anterior pituitary. Am. J. Physiol. **105**: 191. 1933.

Riddle, O., R. W. Bates and S. W. Dykshorn: Thyroid hypertrophy as a response to the gonad-stimulating hormone of the pituitary. Proc. Soc. Exper. Biol. & Med. **30**: 794. 1933a.

Riddle, O. and R. W. Bates: Concerning anterior pituitary hormones. Endocrinology, **17**: 389. 1933.

Turner, C. W. and E. Allen: The normal and experimental development of the mammary gland of the monkey (Macacus rhesus). Anat. Rec. **55**: 80. 1933. (Abst.).

Turner, C. W. and W. U. Gardner: The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agr. Exper. Sta. Bull. 158. 1931.

THE ASCHHEIM-ZONDEK TEST WITH MATURE MICE

A. G. SAR-LOUIS, A.B., M.D., M.Sc. (Med.)
CLEVELAND, OHIO

The work of Hirsch-Hoffmann (1), in which he concluded that the Aschheim-Zondek test could be determined on mature mice 36 hours after injection, was received with considerable skepticism. The question, however, as to whether the test could be performed on mature mice was deemed of such importance that it was decided to repeat the work in order to determine its feasibility. It is rarely important to make a rapid diagnosis of pregnancy and for this reason the number of hours required to read the test was not deemed of importance, but rather its practicability after a maximum time. If, after a hundred hours, it is possible to distinguish the ovaries of a mature or gravid animal injected with pregnancy urine from those injected with urine from non-gravid females, a valuable adjunct to the classical Aschheim-Zondek test has been obtained. The use of mature animals is much more convenient, as young mice weighing from 6 to 8 grams often are difficult to obtain.

In order to become perfectly familiar with the findings in the normal mature or gravid animal, serial sections of many ovaries were made. With each of these series, a section of the vagina also was taken. These sections were carefully examined and the characteristics of each phase of the sexual cycle were noted and correlated with the findings in the vaginal section. It is not necessary to describe here the various psysiologic changes which occur within the ovary, but it is important to emphasize certain findings that later are to be compared with those in the ovaries from animals which received injections of pregnancy urine.

During the estrous phase, the greatest evidence of the stimulating effect of the luteinizing hormone is apparent. The ovaries are larger and more vascularized. The interfollicular stroma is more compressed by the larger size of the luteinizing follicles, by the increase in number and size of the blood vessels and by the increase in the size of the interstitial cells. These latter also partake in the luteinization process. Their protoplasm increases in size and has a greater affinity for the eosin stain. In this phase also the beginning of luteinization of the ruptured follicle may be observed and followed until its completion through the postestrous phase. Hemorrhages into the follicles occur only in early estrus and are seen macroscopically. These hemorrhages (Blutpunkte) are relatively small and may at times occur in a follicle before the ovum is discharged. Most commonly they are seen in a follicle in the epithelium of which luteinization is just beginning. Later, when the luteinization is more advanced, the central hemorrhage has been organized. Luteinization of a follicle begins in the

periphery, spreads and involves simultaneously all of its epithelium. In the control animals, the large cells with acidophilic protoplasm and the very small granulosa cells within the same follicle never were observed. Occasional findings, which may not be entirely normal, but which may be seen in the uninjected animal, are follicle cysts, corpora lutea cysts and corpora lutea atretica.

These findings are mentioned because during the course of our work the article by Brühl and Hollstein (2) appeared, in which these authors concluded that corpora lutea cysts were positive indications of the effect of pregnancy urine. This conclusion could not be substantiated by the studies reported here. In the estrous phase, the ovaries most nearly resemble those of animals which have received injections of pregnancy urine. In the other phases of the cycle are seen only various degrees of development of both corpora lutea and Graafian follicles. Roughly, in

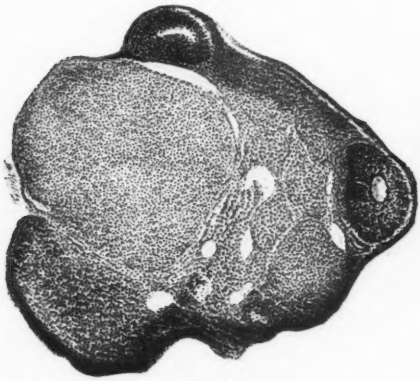

Fig. I

Fig. II

Figure I. Ovary of an adult mouse, injected with pregnancy urine. Intense luteinization of follicles and of interstitial tissue "confluency." (X 100.)

Figure II. Ovary of an adult uninjected mouse in estrus. Two follicles are seen with central hemorrhages (Blutpunkte). Compare the interstitial tissue with Figure 1 and note absence of a "confluency." (X 45.)

diestrum there are mature corpora lutea and young follicles. In proestrum the follicles are more highly developed; they are larger and have larger central cavities. In postestrum two generations of corpora lutea may be distinguished. The young corpora lutea of the preceding cycle are smaller in size and are most easily distinguished from those of older generations by their reactions to the stains. The former are markedly basophilic while the latter are intensely acidophilic. Also, in the younger corpora lutea there is usually a central core of organized hemorrhage. In the older corpora lutea this central core is entirely filled with lutein cells. In the ovaries of the gravid animal are to be found only the large mature corpora lutea and immature follicles.

The injection of pregnancy urine, with its high content of placental hormone, into the mature animal produces very marked and characteristic

changes in the ovaries. As is to be expected, these changes are brought about by an increase in intensity, and an increase in the rate of the luteinization process.

The intensity is particularly expressed in the increase in size, and the acidophilic reaction of the interstitial cells. They become as large as, or at times larger than, those of the young corpora lutea (Figure 1). They have also the same shape, and the same reaction to the stains. As a result, the sharp definition of structures which is so apparent in the normal animals or in those which have received injections of urine from non-gravid women, is lost. The picture is rather that of confluency. Indeed, frequently it is necessary to search for the circular layers of theca to determine the normal boundaries of a corpus luteum. In the center of the

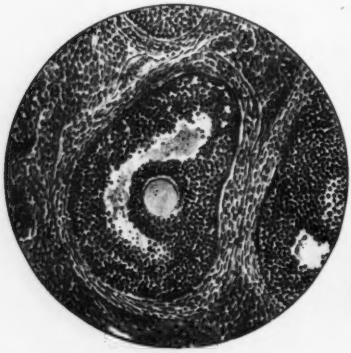

Fig. III

Fig. IV

Figure III. Ovary of adult mouse, injected with pregnancy urine. Luteinization of theca of young follicles. (X 250.)

Figure IV. Ovary of adult mouse, injected with pregnancy urine. Luteinization of follicle. Granulosa cells in centrum and corpora lutea cells in periphery. (X 100.)

section, the luteinized interstitial cells surround the atretic follicles so as to yield an impression of corpora lutea atretica. Confluency was found in the ovaries of all the animals which received injections of pregnancy urine. Whereas in the estrous phase of the control animal there is a certain increase in size of the interstitial cells (Figure 2), this increase is never to be confused with the picture of confluency seen in the ovaries of an animal under the artificial influence of the stimulating hormone in pregnancy urine.

The increased rate of the luteinization process in the animals receiving injections of pregnancy urine is manifested by numerous criteria that are never to be found in the normal animals or those receiving injections of urine from non-gravid females. Often the theca of an immature follicle is luteinized (Figure 3). Often also (in about 90 per cent of the series) there is a mature follicle whose granulosa cells are partially or almost completely luteinized (Figure 4). When partially luteinized, the ovum is surrounded by cumulus cells of the granulosa type, while in the periph-

ery of the follicle the cells are typical of lutein tissue. They are large and their protoplasm has a marked affinity for the eosin stain. When completely luteinized, the follicle is identical with a corpus luteum atreticum. It is an early corpus luteum containing in its centrum a shrunken and devitalized ovum (Figure 5). Another variation found frequently is that of an incompletely luteinized cyst (Figure 6), in which the inner layer of cells is of the granulosa type and the outer layers are of the corpus luteum variety. The presence of two epithelia in one follicle denotes an increased rate of luteinization under the artificial influence of the hormone in pregnancy urine.

These criteria are very striking and easily detected. Other points of difference between the ovaries of uninjected mice and those influenced by pregnancy urine are more quantitative and require for their appreciation study and familiarity with the normal variations. Grossly, the ovaries

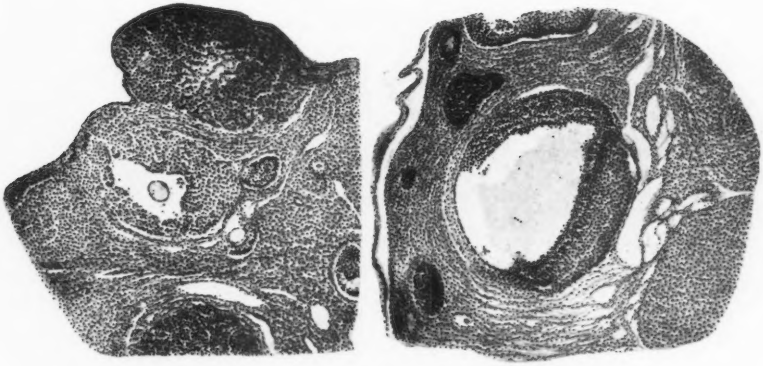

Fig. V Fig. VI

Figure V. Ovary of adult mouse, injected with pregnancy urine. Luteinization of follicle epithelium is complete. Centrum contains atretic ovum. Note also "confluency." (X 100.)

Figure VI. Ovary of adult mouse, injected with pregnancy urine. Corpus luteum cyst with both epithelia present. (X 100.)

influenced by pregnancy urine are larger and the central hemorrhages (Blutpunkte) are increased in size and number. On section, they show a tremendous increase in vascularity. Central hemorrhages are many times larger, and more numerous than those seen in the uninjected animal during pre-estrum and estrus (Figure 7). Moreover, the finding of fresh central hemorrhages in diestrum, late postestrum, and in the gravid state is certainly evidence of increased luteal stimulation. This stimulation, probably through the medium of the anterior pituitary, at a time when this influence normally would be lacking, is evidence of a certain stimulating substance in the test urine. Corpora lutea cysts and corpora lutea atretica are, as stated above, occasionally seen in the uninjected and control animals. Their presence alone, then, in the ovaries of animals treated with pregnancy urine has no significance. Our observations show, however,

that while they appear occasionally in the former, they are numerous in the latter (Figure 8).

TECHNIQUE AND RESULTS

Eight mice received injections of pregnancy urine according to the technique employed by Hirsch-Hoffmann (1), that is, 6 injections of 0.3 cc. each, administered at 4-hour intervals, and were killed 36 to 38 hours after the first injection. In all of this series it was possible to diagnose pregnancy from luteinization of the interstitial cells in the ovaries of the mice. No evidence of increased rate of luteinization was present in the series. Hirsch-Hoffmann's findings that "a beginning change was present in the granulosa cells of mature, often immature follicles, giving them a honey-combed appearance," was not corroborated.

Thirty mice were injected 6 times each with 0.8 cc. at 4-hour intervals and were killed 72 and 100 hours after the first injection. Forty-eight

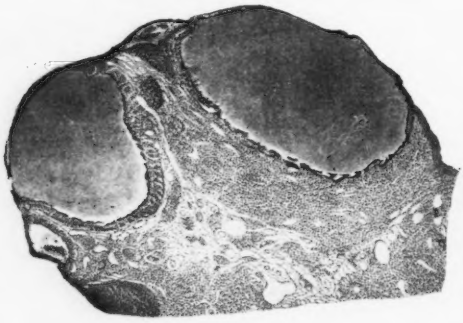

Fig. VII

Fig. VIII

Figure VII. Central hemorrhages (Blutpunkte), as seen in injected animals. Compare size with that in normal animal in estrus as seen in Figure II. (X 45.)

Figure VIII. Ovary of adult injected mouse, showing three corpora lutea atretica, large central hemorrhage, and luteinization of interstitial tissue. (X 45.)

mice were injected 6 times with 0.3 cc. and were killed 72 and 100 hours after the first injection. In all 78 instances, intensity of luteinization as expressed by our term confluency was observed. Seventy of the 78 mice showed many evidences of increased rates of luteinization as expressd by luteinization of theca of immature follicles, by the presence of two types of epithelia in one follicle, etc. No differences were noted in the ovaries of animals killed 72 hours after the first injection and in those of animals killed after 100 hours. Also, no differences were noted with different quantities of urine. Apparently the quantity of the test urine is inconsequential, as so many other more important variable factors exist, over which the investigator has no control.

Fifteen control animals were injected with urine of non-gravid females, receiving six 0.8 cc. injections every 4 hours, and all were killed after 100 hours. In none of this series were any of the criteria observed

indicating the intensity or increased rate of luteinization which were observed in animals which had received injections of pregnancy urine.

After the completion of the routine work the slides made from the sections of ovaries of animals injected with pregnancy urine were mixed with the slides of ovaries from normal and control animals. No difficulty was experienced in making the correct diagnosis in every case.

CONCLUSIONS

The Aschheim-Zondek test may be performed on mature, even gravid animals. The reading of the test may be made after 36 hours, although the evidences of increased intensity and rapidity of luteinization are more striking after 72 to 100 hours.

The increased intensity and rapidity of luteinization are indicated by:

(1) Luteinization of interstitial tissue giving the section a more confluent appearance.

(2) Luteinization of theca of immature follicles.

(3) Partial luteinization of a follicle or cyst so that both granulosa and corpora lutea cells are seen in the same follicle.

(4) Increase in number of corpora lutea atretica and corpora lutea cysts.

(5) Increase in number and size of central hemorrhages.

(6) When the animal is not in the preestrous or estrous phase, the presence of hemorrhages alone is significant.

These changes are so striking that there is no possibility of confusing them with the physiologic changes observed in the ovaries of mice at any stage of the sexual or estrous cycle.

Further experiments have been projected to determine whether there is any quantitative or qualitative difference between the ovarian reaction in mice following injections of the gonadotropic hormone of the anterior pituitary and those observed following the injection of pregnancy urine.

SUMMARY

Adult mice were injected with pregnancy urine in varying amounts. Eight received 6 injections of 0.3 cc. each, every 4 hours and were killed 36 hours after the first injection. Thirty received 6 times 0.8 cc. and were killed, 15 in 72 hours and 15 in 100 hours. Forty-eight received 6 times 0.3 cc. and were killed, 24 in 72 hours and 24 in 100 hours. Serial sections of all ovaries were made and with each series a section of the vagina. The injected series were compared with normal sections from all phases of the ovarian cycle, the vagina being used to determine the phase. Control animals were injected with urine from non-gravid women.

In the 36-hour group the intensity of the luteinization process was indicated by changes in the interstitial tissue. The findings of Hirsch-Hoffman, however, could not be corroborated. No differences were noted

in the ovaries of animals killed after 72 hours from those killed after 100 hours. No differences were noted as a result of varying the quantity of injected urine.

After 72 and 100 hours the changes observed were due to an increase in the rate and an increase in the intensity of the luteinization. The increased rate was detected by (1) luteinization of theca of immature follicles; (2) partial luteinization of a mature follicle; (3) complete luteinization of a follicle producing a corpus luteum atreticum; (4) incompletely luteinized cysts. Similar changes are not seen in any untreated animals at any stage of the ovarian cycle.

The intensity of luteinization was expressed by the involvement of the interstitial tissue. The sharp boundaries of its tissues were lost, giving rise to a confluent appearance. Other changes in the ovaries of the test animals differed only quantitatively from the normal physiologic responses: The organs were larger than normal. Central follicular hemorrhages were much larger and more numerous and were present in certain phases of the cycle in which, under normal conditions, they are absent. These studies indicate that the Aschheim-Zondek test may be made on mature animals. Another series injected with anterior gland substance may show the relationship existing between the gonadotropic hormone of the anterior pituitary gland and the hormone prolan of pregnancy urine.

It is a pleasure to express my thanks to Prof. Oskar Frankl of Vienna, whose kindly assistance and cooperation made this study possible.

REFERENCES

1. Hirsch-Hoffmann, H. U.: Weitere Untersuchungen zur Schwangerschafts-Schnellreaktion an der Geschlechtsreifen und an der Trächtigen Maus. Zum Wesen der beginnenden Umwandlung. Arch. f. Gynäk. **153**: 394. 1933.
2. Brühl, R. and K. Hollstein: Die Schwangerschafts-Schnellreaktion mit Hilfe reifer Mäuse. Arch. f. Gynäk. **154**: 604. 1933.

ENDOMETRIAL STUDIES*

G. S. McCLELLAN, DORIS PHELPS AND JOHN C. BURCH
From the Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine
NASHVILLE, TENNESSEE

It is generally admitted that the endometrium goes through its cycle as a result of ovarian secretion. We now recognize two ovarian secretions, estrin and progestin. The former stimulates the endometrium from the end of one menstruation to just before the beginning of another, while the latter acts in conjunction with the former after ovulation.

Many workers have made worthwhile contributions to the study of ovarian secretion, but our present knowledge of this phase of endocrinology is largely in its infancy due to numerous difficulties. Chief among these is the fact that all the tests clinically applicable depend on a tissue reaction for a measuring standard. The amount necessary to produce these reactions varies so much that the tests can be only roughly quantitative. While not denying the usefulness of these tests, it has seemed to us more reasonable to depend on the patients' own tissue reactions, provided, of course, that sufficient knowledge could be obtained about them so that they could be accurately interpreted. A combination of the two methods, so that the hormone content of the blood and urine, as well as the patients' own tissue reaction, should, of course, give a more complete picture than either method employed singly.

In previous papers (1, 2, 3) we have called attention to several points in interpreting endometrial changes in terms of ovarian activity, and it is the purpose of this paper to extend those studies in the hope of further clarifying the subject. Endometrial hyperplasia is the most important result of ovarian dysfunction, and most of our data have been accumulated to aid in the diagnosis of this most interesting endocrine disorder. It is characterized by profuse and irregular hemorrhages which originate in an endometrium that shows no effect of corpus luteum activity and it is therefore necessary to determine that the profuse and irregular hemorrhages do originate in the aluteal endometrium before making the diagnosis. It is, of course, apparent that sections must be taken at a time when the corpus luteum is active; and we have repeatedly advocated that this should best be taken at the onset of the flow. However, this cannot always be done, and in order properly to evaluate these earlier and later specimens the studies to be reported were undertaken.

These include comparative histological studies of the bleeding hyperplastic and the normally menstruating endometrium, a detailed study of a case of endometrial hyperplasia, in which specimens were obtained fre-

*Read before the Eighteenth Annual Meeting of the Association for the Study of Internal Secretions, Cleveland, Ohio, June 11, 1934.

quently over a long period of time, and an examination of the effects of ovarian hormones upon the endometrium of a castrated woman.

MATERIAL AND METHODS

Tissue for microscopical examination was obtained by the punch biopsy method. This method of obtaining tissue for diagnosis is in constant use in our dispensary and has proved entirely satisfactory. The instrument has previously been described (4) and need not be mentioned in detail. Two specimens from different areas of the endometrium can be obtained at one insertion and the instrument can be used as frequently as desired.

Tissues were fixed in 95 per cent alcohol, Bouin or Regaud fixatives, embedded in paraffin and stained with Erlich's hematoxylin and eosin. Bouin's fluid is by far the most satisfactory fixative for the endometrium. It gives more clear-cut detail and permits of better staining with hematoxylin and eosin.

The estrin content of blood and urine was tested by injection into adult castrated mice, the interval after castration being constant. Whole serum was used; the estrin was extracted from 24-hour specimens of urine by Kurzrok's ethyl acetate method (5) and dissolved in olive oil. Reactions of the vaginal mucosa were determined by microscopic examination as advocated by Fluhmann (6).

COMPARATIVE STUDIES OF THE NORMALLY MENSTRUATING AND THE BLEEDING HYPERPLASTIC ENDOMETRIUM

The material upon which the following comparisons are based includes 20 typical specimens of the bleeding hyperplastic endometrium and 35 specimens taken from normally menstruating women. The latter were distributed as follows:

Day of Menstruation	*Number of Specimens*
1st	11
2nd	15
3rd	6
4th	1
5th	1
2 days post-menstrual	1

All endometrial specimens taken from normally menstruating women showed evidence of progestational changes. The characteristics of the normal endometrium on the first day of menstruation were as follows:

The tissue was relatively scant in amount and consisted chiefly of the basal portion, with usually a few ragged pieces of spongiosa in the process of desquamation. In only two specimens was the compact layer observed and one of these showed a decidual reaction.

The glands were numerous, usually tortuous and most of them were collapsed, although in a few specimens a moderate degree of dilatation was observed. Papillary projections were frequently noted. In height the glandular epithelium was low to tall columnar, the latter predominating

in the basalis. As a general rule, the greater the desquamation in the upper layers, the lower the gland epithelium. The nuclei were usually round or plump oval, basal and widely spaced. There was more tendency toward pseudostratification in the basalis but there was nearly always a distinct layer of cytoplasm above the nuclei. Both nuclei and cytoplasm stained poorly, showing little clear detail of structure. The nuclei in general tended toward pyknosis. Mitoses were not noted. The cytoplasm was pale, homogeneous and mucoid in appearance. No specimens showed the characteristic finely stippled appearance of interval glands, although transitions were noted. There were sometimes relatively large, clear vacuoles which were interpreted as evidence of intracellular secretion; these were only rarely seen in the basalis. The lumen border was indistinct and ragged and gave the impression that the cytoplasm was melting away into the gland lumen. When the section showed low epithelium, it was evident that the majority of the cytoplasm had been lost as secretion. The stroma also exhibited a tendency toward pyknosis of the nuclei and a homogeneous appearance of the cytoplasm. There was an impression of beginning coagulation involving the section as a whole. In none of the specimens was the appearance obtained of actively growing cells.

The material in the gland lumina was mucoid in appearance and concentrated in amorphous masses. Fragmented cellular debris was seldom observed although there were sometimes sheets of relatively intact epithelial cells.

There was no edema and, strange to say, very little hemorrhage. This was not within the stroma except in very few cases but was present at the lumen edge of the tissue.

The characteristics described above were more marked on the succeeding days of menstruation. The first beginning of return to the post-menstrual type was noted on the third day, one specimen showing this condition. On the 4th and 5th days this was more marked, on the second day post-menstrual it was complete.

The characteristics of the bleeding hyperplastic endometrium were as follows: None of the specimens showed any evidence of a progestational reaction. There was usually an abundance of tissue, which consisted principally of the more superficial portions of the endometrium.

The glands showed great variation in form and size, both in the same section and in different sections. All gradations from very small to cystic were seen. They were straight or tortuous, with or without papillary projections, some were dilated and some were collapsed. The presence of cystic glands is not an essential characteristic, as is generally thought. The surface epithelium, when present, was usually pseudostratified, with great crowding of the nuclei. It was similar in structure to that of the glands. The glandular epithelium was usually high, except in the greatly dilated glands, and was pseudostratified, often showing as many as four rows of nuclei. The latter usually occupied the entire depth of the epithe-

lium, leaving no cytoplasmic border toward the lumen. Crowding of the nuclei was frequently extreme, resulting in a slender oval shape. Mitoses were frequently noted. The cytoplasm showed a finely stippled appearance. Both cytoplasm and nuclei stained well, the details of structure were clear and distinct. The lumen border was distinct in spite of the fact that some finely stippled material could often be seen at the tips of the cells, arranged in an irregular manner.

The material in the gland lumina was finely granular and scattered. There were often scattered fragments of cell debris and leucocytes.

The stroma was similar to the glandular epithelium in that the nuclear and cytoplasmic structure was distinct. The cytoplasm was usually in fine filaments giving the stroma a cob-webby appearance. Scattered areas of necrosis were occasionally observed, but these were not extensive. The sections as a whole gave a much more distinct impression of vigorous, living tissue than did the sections of normal endometrium.

There was usually more or less hemorrhage, both intra- and extra-stromal, in general far more than was seen in any of the normal specimens. Edema was the rule and often sheets of coagulated serum and strands of fibrin were present.

The chief differences between the bleeding hyperplastic and the normally menstruating endometrium are summarized in the accompanying table.

If sections are taken near the beginning of bleeding, these differences are more clear-cut than later.

CASE STUDY OF ENDOMETRIAL HYPERPLASIA

The characteristic "swiss-cheese" pattern of endometrial hyperplasia has been considered by many to be a constant feature of the disease. For some time we have maintained that this is not a necessary accompaniment and the case which we are presenting clearly shows that this feature of the disease may be a transitory one. In our case it was present at the onset of observation but was not present during the latter part of the observation period. Moreover, this finding again emphasizes the importance of obtaining specimens at or near the onset of bleeding.

The patient was a colored woman, aged 23, who gave a history of profuse and irregular bleeding for three years. She was observed from January 11 to March 20, 1934. During the period from February 13 to March 20 weekly biopsies were taken and simultaneous determinations of the estrin content of the blood and urine were made.

The first specimens taken from this patient showed a well developed case of endometrial hyperplasia which gave the so-called characteristic "Swiss-cheese" pattern. In later specimens from this same case, this feature was conspicuously absent, although the clinical symptoms were the same at both times. This conclusively shows that this feature is not a universal and constant accompaniment of the disease and that the diagnosis can be made in its absence. In this case the level of the blood estrin

CHART 1.

SUMMARY OF HISTOLOGICAL DIFFERENCE BETWEEN THE NORMALLY MENSTRUATING AND HYPERPLASTIC ENDOMETRIUM

I. General.	Normal.	Hyperplastic.
a. Appearance.	Mucoid. Impression of coagulation.	Impression of vigorous, living tissue. Necrosis, if present, only in small areas.
b. Reaction.	Progestational.	Estrin.
c. Gland form, size, degree of dilatation, number.	Regular, usually numerous.	Irregular.
d. Hemorrhage.	Very little, usually extra-stromal.	Profuse, both intra- and extra-stromal.
e. Edema, fibrin.	Rare.	Frequent.
f. Amount and type of tissue.	Relatively scant, chiefly basalis.	Abundant, chiefly upper layers.
II. Gland epithelium.	Columnar in upper layers. May be pseudostratified in basalis.	Pseudostratified.
a. Nuclei.	Basal, round, widely spaced. Border of cytoplasm toward lumen. No mitoses.	In all positions, forming several rows, slender oval, crowded. Often no border of cytoplasm toward lumen. Frequent mitoses.
b. Cytoplasm.	Homogeneous, mucoid. May contain secretory vacuoles.	Stippled appearance. No secretory vacuoles.
c. Lumen border.	Indistinct and frayed.	Distinct.
Material in gland lumina.	Mucoid, homogeneous, in amorphous masses. Little scattered cell debris but may be sheets of desquamated cells.	Usually scattered, finely granular, often disintegrated cell debris and leucocytes.

rose during the period of bleeding and fell during the interval between bleeding. These changes, while definite, were not of a very marked degree. The growth and activity of the endometrium roughly paralleled the estrin curve. The urinary estrin was almost constant but at one point, close to the end, showed a sharp drop which paralleled endometrial and blood curves.

The curves and method of charting are shown in the accompanying illustration.

ACTION OF THE OVARIAN HORMONES ON THE ENDOMETRIUM OF THE CASTRATED WOMAN

In a previous paper (7) a study of the endometrial changes following the injection of estrin and progestin into castrated rats was reported. Since that time papers have appeared by Werner and Collier (8), Clauberg (9), and Kaufmann (10), who, working on the castrated human female, have reported certain changes in the human as the result of the administration of these hormones. In order to recheck previous work a 40-year-old white woman who had been castrated one year previously was utilized. The hormones used were commercial preparations which were secured from the manufacturer. These were designated by them as Progynon B and Proluton. One cc. of Progynon B contained 50,000 mouse units of an estrogenic hormone, 1 cc. of Proluton contained 5 rabbit units of the progestational hormone.*

*Supplied by the Schering Corporation through the courtesy of Dr. Erwin Schwenk.

Intramuscular injections of 1 cc. each of Progynon were given on April 16, 19, 23, 26 and 30. Bleeding began on May 4. Proluton (2 cc. intramuscularly) was given on May 4, 5, 6, 7 and 8. Bleeding continued until May 13.

This method of administration was recommended by the manufacturer and the appearance of bleeding before the time anticipated by them destroyed much of the value of the experiment. However, it is interesting to note that the growth of the endometrium following a total dosage of 250,000 mouse units was very slight. Bleeding occurred following the cessation of estrin administration and some slight progestational changes were obtained by the use of Proluton even though the patient was bleeding at the time. The histological appearance of the cells was similar in all respects to those seen in the interval and progestational phases.

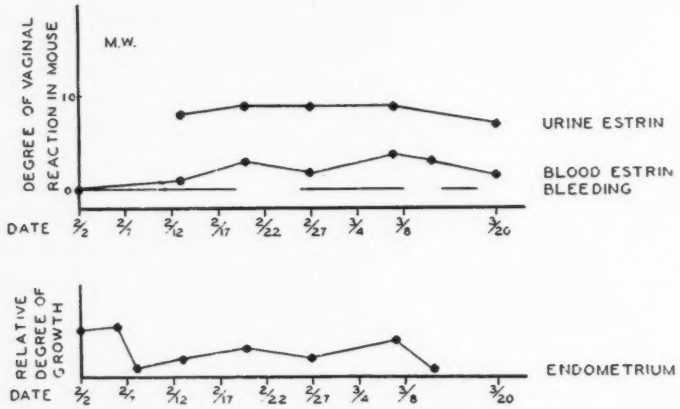

CHART 2

Graphs showing urine estrin level, blood estrin level, duration of bleeding and endometrial activity in a case of endometrial hyperplasia.

Explanation of Chart: In assaying the estrin content of blood and urine, series of injections of ½, ¼ and ⅛ cc. respectively were given. Mucification of the vagina after any series was designated as 1 unit response; stratification after any series, 2 units; cornification after any series, 3 units.

In the line designated "bleeding," the black lines indicate periods of bleeding, blanks indicate non-bleeding intervals.

The curve of endometrial growth is an arbitrary representation of the relative growth present on the date indicated, as judged by comparison of the various specimens.

SUMMARY AND CONCLUSIONS

Three series of experiments dealing with the interpretation of human endometrial changes in terms of ovarian activity have been reported. These include histological studies of the normally menstruating and the bleeding hyperplastic endometrium; the relation of endometrial growth, blood and urine estrin and bleeding periods in a case of endometrial hyperplasia; and the effect on the endometrium of a castrated woman of injections of estrogenic and corpus luteum hormones. Specimens of endometrium in all cases were obtained by the punch biopsy method.

The histological characteristics of 35 specimens of normally menstruating endometrium were compared with those of 25 typical specimens of bleeding hyperplastic endometrium. The normally menstruating endometrium is characterized by progestational changes, evidence of degeneration with no evidence of active, vigorous growth, and by relatively little hemorrhage and little or no edema. The tissue consists chiefly of the basal layer. The bleeding hyperplastic endometrium is characterized by every evidence of vigorous growth and by considerable hemorrhage and edema. It shows no progrestational changes. The tissue is chiefly from the upper layers.

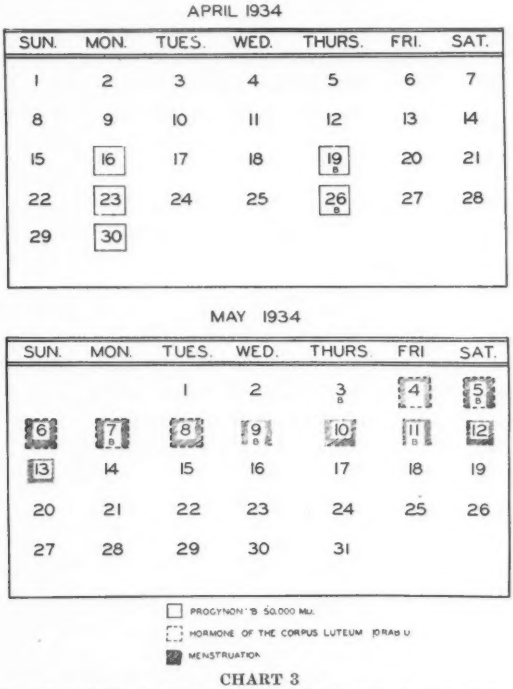

CHART 3

Calendar showing relation of bleeding from endometrium to injections of Progynon and Proluton in a castrated woman.
B indicates biopsies were obtained.

In a case of endometrial hyperplasia, weekly biopsies were obtained over a period of two months. Specimens of blood and urine taken on the days biopsies were obtained were assayed for estrin content. The so-called "Swiss-cheese" pattern was found in specimens taken near the beginning of the observation period but was not found in later specimens. In the interval between bleeding periods and near the onset of the latter, the greatest evidence of endometrial growth was found. The level of blood estrin was highest during these intervals and showed a decline before the onset of the succeeding period of bleeding.

A castrated woman was given a series of injections of estrogenic and corpus luteum hormones over a period of one month. A total of five intramuscular injections of 50,000 mouse units each, administered twice weekly, of estrogenic hormone was followed by five daily injections, intramuscularly, of 10 rabbit units each of corpus luteum hormone. Administration of the latter was begun four days after the last injection of estrogenic hormone. Weekly biopsies were taken throughout the experiment. The estrogenic hormone provoked a mild growth of the endometrium. Bleeding began four days after the last injection of this hormone and continued for 10 days. A slight progestational reaction appeared during the period of injection of the corpus luteum hormone.

1. These studies have demonstrated that the punch biopsy method is a reliable and safe method of obtaining endometrium for clinical and research purposes.

2. Proper use of the data reported will simplify the diagnosis of endometrial hyperplasia and do much to differentiate luteal from aluteal bleeding.

3. It has been clearly shown that the "Swiss-cheese" pattern is not a constant accompaniment of endometrial hyperplasia and that the diagnosis can and should be made in its absence.

4. In the case reported, bleeding followed a decline in the level of blood estrin and checked following a rise in the level of blood estrin.

5. The reactions of the human endometrium are essentially the same as those of the lower animals to injections of estrin and progestin. A dose of 250,000 mouse units provoked a mild growth of the endometrium. The cessation of estrin administration was followed by bleeding.

REFERENCES

1. Burch, J. C., W. L. Williams, J. M. Wolfe and R. S. Cunningham: J. A. M. A. **97**: 1859. 1931.
2. Wolfe, J. M., M. Campbell and J. C. Burch: Proc. Soc. Exper. Biol. & Med. **25**: 1263. 1932.
3. Burch, J. C., D. Phelps and J. M. Wolfe: Arch. Path. In press.
4. Burch, J. C.: J. A. M. A. In press.
5. Kurzrok, R. and S. Ratner: Am. J. Obst. & Gynec. **23**: 689. 1932.
6. Fluhmann, C. F.: Proc. Soc. Exper. Biol. & Med. **31**: 54. 1933.
7. Burch, J. C. W. L. Williams and R. S. Cunningham: Surg. Gynec. & Obst. **53**: 338. 1931.
8. Werner, A. A. and W. D. Collier: J. A. M. A. **100**: 633. 1933.
9. Clauberg, C.: Zentralbl. f. Gynäk. **57**: 1461. 1933.
10. Kaufmann, C.: Klin. Wchnschr. **12**: 1557. 1933.

ACQUIRED RESISTANCE TO THE THYROID-STIMULATING AND PSEUDOLUTEINIZING HORMONE OF CATTLE ANTERIOR PITUITARY*

PAUL MAX, MARY M. SCHMECKEBIER AND LEO LOEB
From the Department of Pathology, Washington University School of Medicine
ST. LOUIS, MO.

Former investigations [Gray and Loeb (1), and Rabinovitch (2, 3, 4)] have shown that administration of potassium iodide to guinea pigs and rats increases considerably the mitotic activity of the acinus cells of the thyroid gland and may produce a slight softening of the colloid, as well as a very slight increase in the size of these acinus cells. The number of phagocytes which invade the colloid becomes much increased. If the oral administration of potassium iodide is interrupted, the gland soon returns to its normal state; the number of mitoses approaches the normal count within three to seven days and after two to four weeks the proliferative activity seems to fall even below normal. However, it was noticed furthermore in our experiments that, when the administration of iodide was continued over a longer time, the phase of maximum stimulation was followed by a period in which the proliferative activity of the acinus cells declined again very markedly, notwithstanding the continued administration of the iodide. The time of the maximum effect and the beginning decline of activities varied with the mode of administration of KI, and differed also in different species. This decline occurred, if the feeding of iodine was continued for four weeks or longer; in this case the colloid accumulated in the acini which thus became large, the pressure exerted by the colloid leading to a flattening out of the walls and to perforations, where the pressure was greatest. We may assume that perhaps also the mitotic activity suffered as the result of the increase in the contents of the acini. After intraperitoneal injections of this substance in the guinea pig Rabinovitch (3) observed a peak in the proliferative activity as early as ten days after the beginning of the injections and a decline between the 15th and 20th day. Also in rats receiving intraperitoneal injections of this substance there was a decline in mitotic activity following a marked increase which reached a maximum as early as five days after the beginning of the injections (4).

Quite analogous observations were subsequently made by us in the case of injections of acid extract of anterior pituitary of cattle in guinea pigs (5). This substance causes, however, a very much more marked proliferation in the thyroid gland than does potassium iodide; furthermore, the structural changes which it produces are very much more pronounced and moreover it was found possible to reproduce, through the administra-

*These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

tion of this substance, all the essential symptoms of severe cases of Graves' disease (6). It was also observed that if the injections of the extract were discontinued the gland returned to its normal state; the number of mitoses fell again and hard colloid began to be secreted anew within the first week. However, this process of retrogression took somewhat longer in this case than after administration of KI, in accordance with the much greater stimulation exerted by anterior pituitary extracts. While after cessation of KI feeding the hypertrophy disappeared within two weeks, some signs of hypertrophy following injection of pituitary extracts remained noticeable for at least three weeks. Yet, in both series the number of mitoses decreased very sharply within a few days after the application of the stimulating substance had been interrupted, and hard colloid began soon to be secreted. This was noticeable as early as three days after cessation of the injections of anterior pituitary extracts.

But, in addition to this retrogression following the cessation of injection, we noticed that even if injections were continued after the full stimulating effect had been accomplished, a gradual retrogression in all activities took place. Notwithstanding the continued administration of this substance, the decline set in at a very early date, as far as certain functions were concerned; thus the number of mitoses reached a maximum in the first few days after the beginning of the injections and then began to decline. Colloid began to accumulate again in at first only slightly larger amounts from about the middle of the third week on. However, at this time the other signs of hypertrophy, such as size of epithelium, irregular shape of acini, were still very pronounced. A complete retrogression was noted usually in six to ten weeks from the beginning of the injections. These retrogressive changes in the thyroid gland were accompanied in the ovaries by the resumption of the normal growth of the follicles and by ovulation and, later on, also by the disappearance of the pseudolutein structures which the thyroid stimulating substance seems to produce in the ovaries. In accordance with the structural changes in the thyroid gland, Siebert and Smith (7) noticed that the basal metabolism gradually declined; but the basal metabolism was usually still somewhat raised at a time when morphologically the gland had almost returned to its normal state.

I. *Earlier experiments concerning the mechanism of the acquired resistance to anterior pituitary extracts.* As to the mechanism underlying this adaptation of the thyroid gland and sex organs to the anterior pituitary extract, we considered the possibility that substances neutralizing the extract might develop in the immune animals and subsequently circulate in the blood. Experiments therefore were carried out in which we obtained the blood serum of guinea pigs which had been injected with the extract over long periods of time and had thus become immune to the effect of the extract (6). We mixed in vitro various quantities of anterior pituitary extract with the serum of the immune animals and injected

the mixtures on successive days intraperitoneally into young guinea pigs varying in their initial weight between 190 and 220 grams. Thyroid as well as sex organs were examined in microscopic sections, the latter in serial sections. We found no specifically inhibiting action of this serum on the effect of the extract either in thyroid or sex organs.

The injections of serum from immune guinea pigs neither prevented the development of very marked hypertrophy in the thyroid gland nor the characteristic changes in the ovaries. However, it is possible that the hypertrophy of the thyroid gland was somewhat more marked in the animals injected with control serum than in those injected with immune serum; but the difference, if it existed at all, was very slight. There is the further possibility that certain, as yet unrecognized variable factors entered into these experiments, and that they were responsible for the negative results we obtained several years ago. Especially in view of the positive results recently obtained by Collip and Anderson in rats, which we shall discuss shortly, it would be advisable to make additional experiments in guinea pigs.

II. *The time of development of the acquired resistance to cattle anterior pituitary extracts in guinea pigs.* We thought it of interest to follow still further the gradual return to a normal state of the thyroid gland of guinea pigs injected with cattle anterior pituitary extracts, giving attention also to the differences in initial weight of the injected animals. It was found that the time extending from twenty-eight to thirty-seven days represents the transition period between marked hypertrophy and marked retrogression. There occur at this time, and sometimes even at a later period, certain individual variations in the rapidity of retrogression. Later on the retrogression becomes more universal, although even then some exceptional cases in which hypertrophic changes are still definite may be observed. As a rule, although not in every guinea pig, a relation is noticeable between the relative gain in weight of an animal and the changes which have taken place in the thyroid gland and in the ovaries; the greater the gain in weight, the more definite was the beginning of a return to normal structure and function in these organs. In the ovaries the increasing resistance to the effects of the injections manifests itself by a resumption of growth in the follicles, as well as by a vacuolar degeneration in the pseudolutein tissue and the gradual disappearance of the pseudolutein bodies and the interstitial gland. We notice also that as a rule the retrogressive changes take a parallel course in the thyroid and in the ovary. While in the majority of the guinea pigs used in this series the initial weight varied between 190 and 210 gms., in a number of animals, injected for periods varying between five and sixteen days, the initial weight ranged approximately between 350 and 400 grams. On the whole the degree of hypertrophy caused by the injections of extract was not very different in the animals with a low and in those with a relatively high initial weight. There was perhaps a slightly greater amount of

colloid found in the thyroids of the animals with a higher initial weight; but the difference even in this respect was not very marked. The amount of extract (1 cc.) injected daily was evidently sufficiently potent to overcome to a large extent the effects which such differences in weight, as occurred in our experiments, might have exerted.

III. *The length of the rest period necessary to cause retrogression in the thyroid gland of guinea pigs previously made hypertrophic through injections of extract of anterior pituitary gland.* In order to have controls in the following series of experiments, we made eleven additional determinations of the changes which take place in the hypertrophic thyroid glands of animals, previously injected with anterior pituitary extracts, during subsequent rest periods. The results of our earlier and of these later determinations are in agreement. After a five-day period of rest, following various periods of effective injections, there was still much hypertrophy, although the number of mitoses was greatly reduced and a considerable amount of hard colloid had again accumulated in the acini. After five or eleven-day periods of injection of the extract, followed by eleven or twelve days of rest, the thyroid had largely returned to its normal state; but there continued to be a slight increase in the size of the acinus cells, in some places. After an eleven-day period of injections, followed by sixteen days of rest, the hypertrophy had almost disappeared, but a slightly greater vacuolization of the colloid persisted. After a five-day period of injections, followed by twenty-two days of rest, we again found an almost normal resting gland, except that in some places there were traces of hypertrophy left.

IV. *Parallelism between the retrogression of the structural changes and of the functional and metabolic effects in the thyroid gland during continued injections of cattle anterior pituitary extracts.* As stated, the same acquired immunity against continued injections of extract of cattle anterior pituitary gland, which was indicated by the return to the normal size and structure of the thyroid gland, by the decrease in mitotic activity and by the accumulation of solid colloid, was also apparent in the decrease in the basal metabolism which took place at a time when the injections were still continued. Thus Siebert and Smith (7), as well as Siebert and Thurston (8), found that, after continued daily injections of 1 cc. acid extract of anterior pituitary gland of cattle, the basal metabolism reached a maximum between the 10th and 12th day; it then either rose very slightly until the 20th day, when it began to fall and reached a level somewhat above normal about four weeks after the beginning of injections, or a gradual decline began from the 12th day on. The period of decline in the basal metabolic rate coincided then approximately with the time when the colloid accumulated again in the acini and this decline continued during the following period. It reached a maximum at a time when the structural signs of hypertrophy began to subside; but there still remained a slight elevation of the basal metabolism, indicating

that there takes place, even at this period, a slightly increased discharge of colloid and thyroid hormone, which is, however, accompanied by an increased accumulation and retention of colloid in the acini. Similarly, Mary M. Schmeckebier (9) found that after long continued injections of acid extract of anterior pituitary gland the stimulating effect on the cells of the adrenal cortex, as indicated by the increase in proliferative activity, began to cease. This effect was presumably exerted directly by the extract on the adrenal gland and was not due to the discharge of the thyroid hormone; the curve, representing the stimulation and the subsequent decline in the proliferative activity of the adrenal gland, takes a course parallel to the changes in structure in the thyroid and to the basal metabolism. A similar decline in the activity of the extract notwithstanding continued injections was found by Holden (10), when he followed the changes which took place in the glycogen content of liver and muscle under the influence of injections of anterior pituitary extract. He found a minimum in liver glycogen six days, and in muscle glycogen eight days, after the beginning of the injections; subsequent to these periods the glycogen content began to rise again. These periods of increased discharge of glycogen correspond approximately to the times when the maximal structural changes are found in the thyroid gland, when the mitotic activity is at a very high level and the colloid has undergone the most marked solution. Following this period an accumulation of glycogen occurred, notwithstanding continued injections of the extract.

V. *Is it possible to prevent or diminish the retrogressive changes in the thyroid gland which take place during continued injections of extracts of anterior pituitary of cattle if, instead of continuous injections, intermittent ones are given?* Soon after we had observed that, during periods of continuous injections of anterior pituitary extracts in guinea pigs, an almost complete retrogression in the hyperactivity of the thyroid gland, as indicated by the structural changes, takes place, and that during a rest period, following the injections, the gland returns almost to its normal state within a relatively short period of time, we began to test the possibility of avoiding such complete retrogression by intermittent injections in which, following a series of injections, the thyroid gland was allowed to regain partly or almost completely its normal equilibrium, during a period of rest, which was again followed by a series of injections. Would the second period of injections produce hypertrophy similar to that produced by the first period or was the return of the gland to its normal state during the period of rest only apparent, while in reality a new equilibrium had been attained either in the gland, or elsewhere in the organism, which caused the result of a second set of injections to be different from that of a first series? In still other experiments we alternated several times between periods of injections and periods of rest. We were of the opinion that the possibility of avoiding the diminution in the effectiveness of the extract, after long continued injections, might be not only of theoretical,

but also of practical interest. We carried out seventeen experiments in which the effect of intermittent injections was studied in sixty-five guinea pigs. In the large majority of cases 1 cc. of the extract each day during the period of injections was given.

We may draw from these experiments the following conclusions: If injections are given discontinuously, periods of injections of different durations alternating with periods of rest varying between ten and thirty days, the gland following the period of rest returns almost to its normal state, except when the period of rest lasted only five days; then the return to the normal state is only partial. If following this period of rest, injections are resumed, there occurs at first after five days again a marked hypertrophy, which is, however, usually not quite so pronounced as after a first five-day period of injections; evidently even in this case the preceding period of injections has had at least a slight and in some instances even a somewhat more marked effect. To some extent, it seems, this slight decrease in hypertrophy can be overcome by an increase in the amount of extract used each time in the second series of injections. However, if in the second series the number of days of injections is increased the hypertrophy usually decreases much more rapidly than if the second series of injections had not been preceded by a first series of injections.

The retrogression may even become more marked than if the first and second series of injections had been given continuously, without interruption by a period of rest. Thus after a second series of injections extending over 11 or 12 days, we find in the majority of cases a marked or moderate reduction in hypertrophy, in contrast to the pronounced hypertrophy observed after a first 11 or 12-day series of injections. For example, after a first and second series of injections both lasting 11 or 12 days, we found in a considerable number of instances a much greater reduction in hypertrophy than after a twenty-two-day series of continuous injections. When the second series extended over sixteen days, there was in four animals a marked, and in one case a moderate, reduction in hypertrophy. If 3, 4, or 6 periods of five days of injections have preceded the last five-day period of injections the latter still led, in some cases, to a marked hypertrophy, but in several other animals the reduction in hypertrophy was more pronounced; evidently the greater frequency of periods of injections preceding the last series has in some instances exerted a gradually cumulative effect, tending to reduce the hypertrophy in the last series. Otherwise the length of the first series of injections or the duration of the rest periods did not seem to be of great importance within the ranges used in these experiments. It would be of interest to extend the rest period beyond thirty days, which is the longest time allowed so far in our experiments.

We may then conclude that instead of continuing the stimulating effect on the thyroid gland, the alternation between periods of injections and of rest, during which the gland was expected to recover at least partly,

did not, on the whole, lead to a prolonged maintenance of hypertrophy, but, on the contrary, seemed to intensify the tendency of the thyroid gland to return to its resting stage and to lose its responsiveness to stimulation. An exception to this result may be noticed, however, when the final series lasted only five days; in this case the hypertrophy was usually marked. It was thus possible in Experiment XVII to obtain in several animals a moderate or marked hypertrophy in guinea pigs which had received altogether forty injections of 1 cc. extract. There would have been, in all probability, a more marked reduction of hypertrophy in the thyroid glands of these animals if the injections had been given continuously instead of intermittently. We may, therefore, conclude that within the ranges of alternating periods of rest and injections used in these experiments the ultimate reduction in the stimulating effects of anterior pituitary extract cannot be prevented, and that on the contrary it may be intensified.

In order to make these conclusions more evident, we shall arrange the different experiments carried out, in three groups differing in respect to the length of the last series of injections.

A. *Experiments in which the length of the last period of injections was five days.**

Experiment I. 5d I.—11d R.—5d I. Six animals. Marked hypertrophy, although there was a slight diminution in effects of extract.

Experiment II. 5d I.—22d R.—5d I. Four animals. Marked hypertrophy, but slight diminution in the effects of the extract.

Experiment III. 11d I.—12d R.—5d I. Three animals. Marked hypertrophy; perhaps some slight diminution in effectiveness of extract.

Experiment IV. 11d I.—12d R.—5d I. (3 cc. extract). One animal. Marked hypertrophy.

Experiment IX. 11d I.—30d R.—5 d I. (3 cc. extract). One animal. Very marked hypertrophy.

Experiment X. 16d I.—30d R.—5d I. Three animals. Moderate to marked hypertrophy; somewhat greater reduction in effects of extract.

Experiment XI. 16d I.—30d R.—5d I. (3 cc. extract). One animal. Very marked hypertrophy.

Experiment XIV. 25d I.—10, 20 or 30d R.—5d I. Three animals. Marked hypertrophy; but slight reduction in effectiveness of extract.

Experiment XVI. Four or five periods of injections lasting five days. Rest periods of 5, 8, or 10 days. Five animals. In three guinea pigs marked although slightly reduced hypertrophy; in one case marked, in another a moderate reduction in hypertrophy.

Experiment XVII. Either five periods of injections, each lasting 8 days and four periods of five days of rest, or 8 periods of injections each

*The following abbrevations are used: d=days. I=injections. One injection was being given daily. R=rest period. 1, 2, or 3cc. signifies amounts of extract injected daily in the second series; in the first series the amounts injected were always 1cc. of the acid extract.

lasting five days, alternating with five-day rest periods. Four animals. Moderate or marked hypertrophy in three animals; in one guinea pig almost a resting state of thyroid.

B. *Experiments in which the final period of injections lasted eleven or twelve days.*

Experiment V. 11d I.—12d R.—11d I. Nine animals. In seven guinea pigs great or moderate reduction in hypertrophy; in only two guinea pigs much hypertrophy with slight reduction.

Experiment VI. 11d I.—12d R.—11d I. (2 cc.). Two animals; in one case marked hypertrophy; in the second, much reduction.

Experiment VII. 11d I.—16d R.—11d I. Eight animals. In five cases almost complete return to the normal state; in two cases very moderate hypertrophy; only in one case definite, though reduced hypertrophy.

Experiment VIII. 11d I.—30d R.—11d I. (2 cc.). Two animals; in one much reduction in hypertrophy; in the second animal very moderate hypertrophy.

Experiment XII. 16d I.—10, 20 or 30d R.—11 or 12d I. Four animals, in two cases slight, in the two others, marked reduction in hypertrophy.

Experiment XV. 25d I.—10, 20, or 30d R.—11d I. Four animals; in two cases marked, although slightly reduced hypertrophy, in the other two, marked reduction.

C. *Experiment in which the last period of injection was 16 days.*

Experiment XIII. 16d I.—10, 20 or 30d R.—16d I. Five animals; in four cases marked reduction, in one case moderate reduction in hypertrophy.

VI. *The mechanisms underlying the acquired resistance to continued injections of extract of cattle anterior pituitary gland.* The observations we made concerning the acquired resistance to the effects of extracts of anterior pituitary of cattle which is observed in guinea pigs, when the injections are continued over a long period of time, have more recently been extended to other species. Thus Collip and Anderson (11) noted that also in the rat the thyroid-stimulating effects of the extract declined with long continued injections. These investigators used the action which the extracts have on the basal metabolic rate as an indicator of their efficiency. They found that with continued injections the basal metabolism not only returned to its normal level, but that it declined as much as 29 per cent below this point. It is of interest in this connection that Rabinovitch found indications that after a long continued administration of KI the proliferative activity of the gland may decline even below the normal level. As was the case in experiments of Siebert and Smith with guinea pigs (7), Collip and Anderson noted a decrease in basal metabolism at a

time when the thyroid gland of the injected rats still showed signs of hypertrophy. Also in rabbits a similar increase in resistance to continued injections of anterior pituitary substances was found by Herz and Kranes (12). They observed that injections of emulsions of extracts of anterior pituitary gland of cattle during periods of from two to seven days produced marked hypertrophy, but that on continuation of these injections the colloid accumulated again in the acini, the acini enlarged and their epithelium became flat. These changes are similar to those which we have observed in the thyroid glands of guinea pigs after long continued injections of ioidine.

As to the mechanism underlying this acquired resistance to the injections of extracts of cattle anterior pituitary glands, Collip and Anderson found that the blood serum, as well as extracts of liver and spleen of rats made resistant to the extract, was able to inhibit the effects of extracts on basal metabolism in rats and also in guinea pigs. They concluded, therefore, that in the serum and in certain organs of rats, injected with extract for a long period of time, substances are present which counteract the effects of the extract, whereas in the serum of injected guinea pigs we had not been able to demonstrate the presence of such substances, and thus to prevent the development of the typical changes in the thyroid gland of the injected animals. While our negative results may have been due to certain factors in the procedure which we used, we must also consider the possibility that in rats the phase of active immunity against the extract becomes more pronounced than in guinea pigs. This may perhaps be connected with the greater resistance which the thyroid gland of rats offers to the stimulating effects of anterior pituitary extracts, as compared with the thyroid gland of guinea pigs, to judge from the intensity of the structural changes which take place in this organ [Loeb and Bassett (13) and Thurston (14)]. It is therefore conceivable that in the rat a negative phase of resistance following a first phase of stimulation has a better chance to develop than in the guinea pig.

If we may then accept the conclusion that the production of neutralizing substances may develop in animals injected with anterior pituitary extract for an extended time, we must ask whether the formation of immune substances is in itself sufficient to explain the state of resistance which follows such long continued injections, or whether in addition other factors enter into this mechanism. There are several facts which make the second alternative the more probable one. They are as follows: (1) The curve of retrogression is different in different kinds of reaction to the injection of extract. Thus the proliferative activity of the acinus cells, as stated, reaches a maximum in the first few days following injections; the colloid begins to increase somewhat in amount as a rule between the 16th and 20th day, at a time when the epithelial cells are still very hypertrophic. Now the relative diminution in mitotic activity, which begins at so early a date, must be due to mechanisms other than the formation of

immune substances. (2) The curves of stimulation and decline of stimulation in the thyroid gland of the guinea pig are very similar in experiments in which anterior pituitary extract and in those in which KI was used as stimulant; the differences which exist are to a large extent of a quantitative nature. We should, therefore, expect that similar factors are responsible for the development of a phase of resistance in the case of both of these substances. It would be difficult to assume that, after long continued administration of so simple a substance as KI, neutralizing immune substances begin to circulate in the blood of the treated animals; it is much more probable that other mechanisms, presumably residing in the thyroid gland itself, are largely responsible for the acquired resistance.* (3) In the rat the maximum in the stimulating action of iodine is reached as early as five days after the beginning of the injections and following this stage a rapid decline sets in; it would be difficult to conclude that a correspondingly rapid development of immune substances is responsible for this phenomenon. (4) There is known already a mechanism which tends to counteract the stimulating influence of the anterior pituitary hormone, but it does not depend on the production of a substance neutralizing the extract in vitro. As a result of the stimulation of the thyroid gland, caused by the injection of the anterior pituitary extract, an increased amount of thyroid hormone is produced and given off into the circulation. This hormone lowers the level of reactivity of the thyroid gland to various stimuli, such as the removal of a portion of this organ [otherwise sufficient to induce compensatory hypertrophy (15)], administration of KI (16) and injection of anterior pituitary extract (17). Thus a balancing, self-regulating mechanism is produced which, by reducing the responsiveness of the thyroid gland to numerous stimuli, counteracts the anterior pituitary effect. We may assume that this mechanism will be active also in experiments such as those with which we deal in this paper. The thyroid hormone would then have a twofold function; in the first place it causes an increase in certain metabolic and functional activities and in the second place it tends also to produce a state of rest in the thyroid gland and thus inhibit the production of the thyroid hormone. (5) A somewhat related phenomenon, to the one we have described in the case of the thyroid gland of the guinea pig, has been observed in mouse tumors by Fleisher, Vera and Loeb (18) and Fleisher and Loeb (19). These investigators found that the resistance to the action of hirudin and colloidal copper acquired is due to a double mechanism, namely, (a) to the development of a purely cellular resistance residing in the tumor cells, and (b) to the development, in the bearer of the tumors, of substances or mechanisms neutralizing the effect of the injected substances. It seems probable that also after injections of anterior pituitary extracts we may have to deal with a double mechanism leading to resistance to this sub-

*Quite recently A. Loeser and K. W. Thompson (Endokrinologie **15**: 144. 1934), found indications that iodine exerts its effects on the thyroid gland by way of the anterior pituitary. Without doubting the correctness of their observations, the latter may perhaps admit of a different interpretation.

stance, one residing in the thyroid gland itself and the other one due to the production of antagonistic substances elsewhere in the body. As far as the effects of intermittent injections of anterior pituitary extracts are concerned, these may be attributed to either of these two mechanisms and the observations cited do not, therefore, make possible a decision between these two possibilities.

There remains still the question as to whether the effects of increased resistance, observed after injections of cattle anterior pituitary extracts, would apply also in case the animal's own anterior pituitatry gland should produce an excessive amount of hormone? There is no indication that the constant action of the anterior pituitary hormone leads to the production of specific substances circulating in the blood and able to neutralize the hormone, which would thus act as antigen. Inasmuch as it is known that extirpation of the anterior pituitary gland in rats leads to an atrophy of the thyroid gland and to a reduction in basal metabolism [P. E. Smith and Foster (20)], we may assume that the anterior pituitary exerts a constant stimulating action on the thyroid. It is probable that the substances neutralizing the anterior pituitary hormone are directed primarily against the heterogenous protein which forms a part of the anterior pituitary hormone and only secondarily and indirectly they affect the hormone action as such. It is therefore not probable that if in Graves' disease an excessive overstimulation of the thyroid gland, by a hormone of the anterior pituitary of the individual affected by this disease, should occur, this process would lead to the production of neutralizing antibodies in the organism. On the other hand, it is conceivable that such an overactivity of the anterior pituitary might lead to the development of adaptive changes in the thyroid gland itself and thus to a mitigation or possibly to a temporary remission of the disease. As stated, the increased production of thyroid hormone as such would tend to have such an effect. In former investigations when we pointed out that it is possible through continued injections of extract of cattle anterior pituitary gland to imitate the essential symptoms of Graves' disease, we also stated that the occurrence of a complete or an almost complete refractory state of the thyroid gland to the stimulating effects of these extracts developing after long continued injections, might perhaps be considered as an objection to the conclusion that the anterior pituitary gland is involved in the etiology of this disease. However, at that time we also pointed out that the development of such a state of resistance might perhaps explain the remissions which may occur in the course of this disease. Yet, there would still remain the problem, why an acquired state of resistance would not be permanent and lead to a spontaneous cure. However, if we consider the probability that the development of neutralizing substances, circulating in the blood of an animal injected with cattle anterior pituitary extracts, is not primarily due to the presence of an excessive amount of hormone, but to the action of a foreign protein, this problem loses much of its importance.

SUMMARY

When guinea pigs are injected with the thyroid-stimulating hormone of the anterior pituitary gland over long periods of time, a first period of stimulation in the thyroid and the production of characteristic changes in the ovary is followed by a second period of retrogression and return to the normal state, which takes place notwithstanding the continued injections. This retrogression affects the structure (growth), as well as the various functions exerted by the anterior pituitary hormone and by the organs on which it acts. The curves of stimulation and retrogression differ in the case of different structural and functional conditions, but it is possible to coordinate certain structural with definite functional effects of the hormone. The period from the fourth to the sixth week following the beginning of injections of 1 cc. of cattle anterior pituitary extract seems to be the time of transition from the state of stimulation to the refractory state.

Inasmuch as after a rest period, during which administration of the extract is interrupted, the thyroid gland soon returns to an almost normal condition, it was considered possible that if a retrogression has thus been achieved the gland might respond to new injections of the extract with the typical stimulation and that it might thus be possible to avoid the refractory state. However, it was found that the gland responds to the second set of injections only with a transitory period of stimulation, which is followed by a more rapid development of the refractory state than that developing after a first continuous series of injections. It has therefore not been found feasible by these means to avoid the gradual development of a refractory state in the animal submitted to the effects of the hormone over a considerable period of time.

As to the mechanism underlying the development of the refractory state, it seems probable that the latter is due not only to the formation of substances neutralizing the extract, which may take place in the injected animal, but also to other factors, such as the antagonistic relation between the thyroid-stimulating anterior pituitary hormone and the thyroid hormone. Furthermore, it appears probable that the development of the neutralizing substances is caused primarily by the injection of foreign protein—acting perhaps as carrier—rather than of the hormone as such and that the neutralizing effect on the hormone is therefore of an indirect nature.

REFERENCES

1. Gray, S. H. and Leo Loeb: Am. J. Path. **4**: 257. 1928.
2. Rabinovitch, J.: Am. J. Path. **4**: 601. 1928.
3. Rabinovitch, J.: Am. J. Path. **5**: 91. 1929.
4. Rabinovitch, J.: Proc. Soc. Exper. Biol. & Med. **28**: 394. 1931.
5. Loeb, Leo and Hilda Friedman: Proc. Soc. Exper. Biol. & Med. **29**: 172. 1931.
6. For references see: Loeb, Leo: Klin. Wchnschr. **11**: 2121; 2156. 1932.
7. Siebert, W. J. and R. S. Smith: Proc. Soc. Exper. Biol. & Med. **27**: 622. 1930. Am. J. Physiol. **93**: 376 1930.
8. Siebert, W. J. and E. W. Thurston: Proc. Soc. Exper. Biol. & Med. **29**: 652. 1932.
9. Schmeckebier, Mary M.: Proc. Soc. Exper. Biol. & Med. **31**: 770. 1934.
10. Holden, Raymond F., Jr.: Proc. Soc. Exper. Biol. & Med. **31**: 773. 1934.

11. Collip, J. B. and E. M. Anderson: Lancet, **226**: 76. 1934.
12. Hertz, S. and A. Kranes: Endocrinology, **18**: 415. 1934.
13. Loeb, Leo and R. B. Bassett: Proc. Soc. Exper. Biol. & Med. **26**: 860. 1929. **27**: 490. 1930.
14. Thurston, E. W.: Arch. Path. **15**: 67. 1933.
15. Loeb, Leo: J. Med. Research, **41**: 481. 1920.
16. Gray, S. H. and J. Rabinovitch: Am. J. Path. **5**: 485. 1929.
17. Loeb, Leo, R. B. Bassett and Hilda Friedman: Proc. Soc. Exper. Biol. & Med. **28**: 209. 1930.
18. Fleisher, M. S., Miguel Vera and Leo Loeb: J. Exper. Med **20**: 522. 1914.
19. Fleisher, M. S. and Leo Loeb: J. Exper. Med. **21**: 155. 1915.
20. (a) Smith, P. S.: J. A. M. A. **88**: 158. 1927; (b) G. L. Foster and P. E. Smith, J. A. M. A. **87**: 2151. 1926; (c) P. E. Smith, C. F. Greenwood and G. L. Foster: Am. J. Path. **3**: 669. 1927.

THE RELATION OF BLOOD VOLUME TO CERTAIN GLANDS OF INTERNAL SECRETION. EFFECT OF THYROID AND GONAD ABLATION

MAE FRIEDLANDER, Ph.D., NORMAN LASKEY, M.D.
AND
SAMUEL SILBERT, M.D.
From the Laboratories and Out-Patient Department of The Mount Sinai Hospital
NEW YORK

A striking reduction of blood volume is present in individuals with thrombo-angiitis obliterans (1). The change is due to a reduction of whole blood, although the decrease in plasma is probably slightly greater than that of the cells. In anemias and myxedema a reduction has also been noted, but in these cases the plasma volume is relatively normal, and this reduction of total volume is due to a diminished number of cells (2). A reduced blood volume following bilateral oöphorectomy in women and animals has been reported (3). Together with the reduction in blood volume there is found an increase in certain chemical constituents of the blood. These changes closely resemble those found in the blood of individuals suffering with thrombo-angiitis obliterans. In spite of these alterations, no serious clinical manifestations have been noted in these bilaterally oöphorectomized women.

Stimulated by the work of Thompson, who showed that in myxedema cases an increase of blood volume could be produced by the use of thyroid extract (2), we treated thrombo-angiitis obliterans cases with thyroid and noted a rapid increase in blood volume (4). We therefore felt that the relationship of thyroid activity to blood volume was worthy of further experimental study. We performed thyroidectomies on male and female cats in order to determine the effect of this procedure. Since striking differences were noted in the two sexes, we investigated the effect of the removal of the gonads. Differences were also observed in the two sexes following gonadectomy.

EXPERIMENTAL MATERIAL AND METHODS

Cats were employed for this study. Blood volume and chemical studies were made and checked before operation, and then repeated about every four weeks after operation. With the animals under mild ether anesthesia it was not difficult to enter the external jugular veins and make accurate determinations. Blood volume determinations were made by the Lampe dye method modified by Rosenthal. Bloor's method was used for the cholesterol determinations and Wu's method for fibrinogen.

The thyroidectomy operation consisted of almost total removal of the gland. In the cat the parathyroid bodies are attached to the poles of the thyroid and a small fragment of tissue had to be left to avoid death by

tetany. The removal of the gonads presented no difficulties. Of about 70 animals used for this investigation, 35 lived long enough to allow repeated tests to be made.

RESULTS

Thyroidectomy produced a sharp reduction in blood volume in 19 male animals. Ten typical examples are presented in Table 1. Within a

TABLE 1

BLOOD VOLUME AND CHEMISTRY BEFORE AND AFTER THYROIDECTOMY IN 10 MALE CATS

MALE CATS

No.	Date	Blood Volume in cc. per kg.	Plasma Per Cent	Weight in Kgm.	Cholesterol	Fibrinogen
5	1/19/32	67	57	2.5	80	2.
	3/ 5/32	45	68	2.9	110	3.
14	6/24/33	68	69	2.2	80	2.
	1/ 2/34	55	54	2.7	100	2.2
16	8/30/32	80	70	3.1	80	1.9
	1/11/33	54	55	3.2	150	3.
23	12/31/31	65	65	3.8	80	2.
	4/13/32	41	67	4.1	135	3.
33	7/12/32	75	60	1.6	80	1.9
	9/ 1/32	65	65	1.9	110	2.2
35	7/12/32	79	60	2.	80	2.
	1/12/33	61	70	2.1	110	3.
36	9/ 1/32	50	62	3.2	80	2.
	5/ 9/33	48	55	3.9	110	2.2
38	7/13/32	66	60	2.5	75	2.
	11/21/32	50	68	3.1	100	2.1
40	8/30/32	73	68	3.5	...	1.8
	1/23/33	50	66	3.5	120	2.5
67	10/31/33	60	60	3.5	70	1.8
	1/26/34	41	67	4.5	90	2.1

few weeks after operation, reduction of blood volume was noted, and subsequent tests revealed that the low volume persisted. In order to simplify the table, intermediate determinations are omitted, and only the first figures before operation and the last after operation are given. In all of these animals the reduction in blood volume was striking, and with a single exception there was no tendency to return to normal in spite of a period of observation as long as nine months. In one case in which the reduction of blood volume was followed by a gradual return to normal, post mortem examination failed to reveal any evidence of regeneration of thyroid tissue. The only clue to this discrepancy may be the fact that the animal was immature at the time of operation. Otherwise the results are strikingly consistent and indicate that the removal of the thyroid in male cats is followed by a sharp reduction in blood volume. The chemical changes in the blood show consistent elevation of cholesterol and fibrinogen.

An opportunity presented itself for study of the blood volume in one male patient who had had a total thyroidectomy about a year ago for an

advanced cardiac disorder. This patient was in good condition at the time of the test and his blood volume was 61 cc. per kg. The normal figure is about 80 to 85 cc. per kg.

Since thrombo-angiitis obliterans occurs almost exclusively in males, we were primarily interested in the blood volume changes in this sex. On this account the number of female cats operated upon was considerably less. Thyroidectomy in five female cats, in sharp contrast to the males, was followed by no change in blood volume. One of these animals was observed for 14 months, and another for 10 months after operation. The cholesterol and fibrinogen figures remained relatively unchanged on repeated examinations.

TABLE 2

BLOOD VOLUME AND CHEMISTRY BEFORE AND AFTER THYROIDECTOMY IN 5 FEMALE CATS

FEMALE CATS

No.	Date	Blood Volume in cc. per kg.	Plasma Per Cent	Weight in Kgm.	Cholesterol	Fibrinogen
5A	3/13/32	67	66	2.	80	2.1
	5/ 5/33	65	58	2.7	90	2.1
10	5/12/32	64	60	2.6	85	2.
	3/ 8/33	60	60	2.8	110	2.4
15	12/ 9/31	65	65	2.4	70	2.
	3/10/32	72	70	2.2	90	2.2
17	10/17/33	47	60	3.	90	1.8
	2/13/34	50	60	3.1	90	1.9
37	7/13/32	70	64	2.3	80	2.
	9/ 7/32	70	65	2.5	70	2.

Bilateral oöphorectomy in five cats was followed by a striking fall in blood volume (Table 2). Since bilateral oöphorectomy is frequently performed in women an opportunity to study changes in the blood volume of such patients was readily obtained. The results of such a study in 25

TABLE 3

BLOOD VOLUME AND CHEMISTRY IN SIX FEMALE CATS BEFORE AND AFTER BILATERAL OOPHORECTOMIES

No.	Date	Blood Volume in cc. per kg.	Plasma Per Cent	Weight in Kgm.	Cholesterol	Fibrinogen
69	6/19/33	73	60	3.5	70	1.8
	1/ 5/34	48	56	3.4	100	2.
39	6/18/33	74	62	2.6	90	2.
	12/28/33	50	57	3.2	110	2.
37A	4/12/33	64	60	2.7	75	2.
	12/19/33	42	68	3.3	110	2.1
37	9/ 7/32	70	64	2.5	70	2.
	2/28/33	40	60	3.3	120	2.5
*29	9/27/32	60	64	2.4	70	2.
	5/ 9/33	68	61	2.2	80	1.9
5A	5/ 5/33	65	58	2.7	90	2.1
	1/ 2/34	50	60	3.	110	2.

*Left oophorectomy only.

women from whom both ovaries had been removed revealed similar changes (3). Therefore from the animal and the human studies that have been made the conclusions may be drawn that bilateral oöphorectomy is followed by a striking reduction in blood volume.

No effect was produced upon the blood volume in cats in which bilateral orchidectomy was performed (Table 4). Opportunity to study patients with bilateral orchidectomy is exceedingly rare. Fortunately, one such patient was available and two blood volume determinations two years apart revealed a normal volume. Thus it may be stated that the effects on blood volume of removal of male gonads is in sharp contrast to castration in the female.

TABLE 4

BLOOD VOLUME AND CHEMISTRY BEFORE AND AFTER BILATERAL ORCHIDECTOMIES IN 5 MALE CATS

MALE CATS

No.	Date	Blood Volume in cc. per kg.	Plasma Per Cent	Weight in Kgm.	Cholesterol	Fibrinogen
40A	4/26/33	50	65	3.1	90	2.
	1/ 3/34	51	64	3.8	90	1.9
38	4/10/33	65	55	2.5	80	2.
	4/12/34	60	58	4.	90	1.9
5	6/18/34	77	60	2.7	80	1.8
	9/12/34	80	64	2.2	80	2.
15	6/ 6/34	51	60	3.	100	1.8
	9/12/34	51	60	3.6	100	1.9
14	7/16/34	50	59	4.6	95	2.
	9/ 7/34	55	64	4.3	80	1.8

DISCUSSION

Hammett (5, 6) suggests that the inter-relationship of the glands of internal secretion differs in the two sexes. The observations reported here bring additional support to this view. It seems clear that the thyroid gland exerts a controlling influence on the blood volume in the male, and that a similar rôle is played by the ovaries in the female. Whether in turn this control is affected by way of the adrenal glands remains to be shown. It seems possible that a study of their relationship may afford a clue as to the peculiar immunity enjoyed by the female against thrombo-angiitis obliterans.

SUMMARY AND CONCLUSIONS

Blood volume and chemistry studies were made before and after operation in 35 cats. A striking reduction in blood volume was noted in 19 male cats after thyroidectomy. No changes in blood volume or chemistry were noted in five female cats following thyroidectomy. A striking reduction in blood volume followed the removal of both ovaries in five cats. Bilateral orchidectomy in five cats produced no change in blood volume.

Corresponding results were obtained in human patients following similar operations.

REFERENCES

1. Silbert, S., A. L. Kornsweig and M. Friedlander: Studies in thrombo-angiitis obliterans (Buerger) IV. Reduction of blood volume, Arch. Int. Med. **45**: 948. 1930.
2. Thompson, W. O.: Studies in blood volume. I Blood volume in myxedema, with comparative plasma volume changes in myxedema and cardiac edema. J. Clin. Investigation, **2**: 477. 1926.
3. Friedlander, M., N. Laskey, S. Silbert: Reduction of blood volume following bilateral oöphorectomy. Proc. Soc. Exper. Biol. & Med. **30**: 1263. 1933.
4. Friedlander, M. and S. Silbert: Thrombo-angiitis obliterans (Buerger) VIII. Effect of thyroid administration on blood volume. J. A. M. A. **97**: 17. 1931.
5. Hammett, F. S.: Studies of the thyroid apparatus. X. Refractive index and water content of serum of male and female albino rats thyro-parathyroidectomized at 100 days of age. Am. J. Physiol. **64**: 467. 1923.
6. Hammett, F. S.: A biometrical study of the size and inter-relationship of the glands of internal secretion. J. Metab. Research, **7-8**: 91. 1925-26.
7. Rowntree, L. G. and G. E. Brown: The Volume of the Blood in Health and Disease. W. B. Saunders Company, Philadelphia, 1929.

THE EFFECT OF SPAYING ON BODY GROWTH AND THE ORGAN WEIGHTS OF THE ALBINO RAT

CLAY B. FREUDENBERGER AND OSCAR A. BILLETER
Department of Anatomy, University of Utah
SALT LAKE CITY

INTRODUCTION

It seems of great interest at the present time to know the exact results of removal of the ovaries since so much work is being done on the ovarian hormones. In the experiment presented here, it seemed desirable to extend observations over a larger number of organs than had been done heretofore.

A number of workers between the years 1900 and 1910 were engaged in spaying experiments. Much of their work may be criticized on the grounds that most of their experiments involved only a small number of test animals with inadequate controls. No attempt was made by these investigators to include observations on a maximal number of organs, particularly the abdominal and thoracic viscera. The only experiments comprising a wider range of data are those of Marassini and Luciani (1911), Hatai (1913, 1915), and Livingston (1916). To the publications of the latter two authors reference is made for a review of most of the literature up to their time. None of these authors applied modern biometrical methods to their data.

Recently, Anderson and Kennedy (1933), working also with albino rats, performed a gonadectomy experiment in an attempt to clear up the many contradictory results obtained by previous workers concerning the changes in the hypophysis, suprarenal glands, and thyroid following this operation. They review the literature for these three organs.

MATERIALS AND METHODS

The rats used in our work were of the Wistar strain. At the time of birth all litters were reduced to six and weaned when three weeks old. They were operated upon at an average age of 26 days, ranging from 23 to 29 days for the controls, and 24 to 29 days for the test animals. Whenever possible, as was the case in most instances, a test animal was controlled by one of the same litter of approximately the same weight. The average weight at the time of operation was 47 grams, being the same for the spayed as for the controls. The majority of the animals seemed to be in a good state of health throughout the period. A constant water and food supply was furnished, the diet consisting of: whole ground wheat, 67.5 per cent; casein, 15 per cent; whole milk powder, 10 per cent; sodium chloride, 0.8 per cent; calcium carbonate, 1.5 per cent; and butter, 5.2 per cent, to which a small amount of cod liver oil was added.

The ovaries were removed through a midline abdominal incision, the rats being under ether anesthesia. The control animals were treated in a

like manner except that their ovaries were not removed. Recovery from the operation was good in all instances. All the animals were weighed weekly. At an average age of 184 days, ranging from 178 to 188 for the test animals, and from 180 to 188 for the controls, the rats were killed with chloroform or in a gas chamber. They were then immediately autopsied and their organs weighed. The method of autopsy was in general the same as that used by Jackson (1930). The "alimentary group" was weighed together, consisting of the stomach and intestines with their contents, mesentery and pancreas. Then the mesentery, pancreas, and contents were removed, and the empty stomach and intestines were weighed separately. The heart was opened to remove all blood clots.

The experiment comprised two series, the first one consisting of 12 test animals and 11 controls, the second of 13 test animals and 14 controls. The first group was born on days ranging from October 25 to November 11, so that the period of most intense growth occurred in the winter months, whereas the second series was born on days ranging from April 11 to June 18, so that their main period of growth occurred in the summer. This seasonal difference is emphasized, as the two groups, although originating from the same stock, show a striking difference, the winter group (first series) presenting much more marked growth changes than the second group. Due to inability to maintain equal temperature and humidity throughout the year, the second group suffered considerably from the heat. This difference between the two groups may in part, as will be discussed later, explain some of the discrepancies found in the literature regarding various changes following gonadectomy.

To test the reliability of the differences obtained, the difference between the two means was divided by the probable error of the difference, thus obtaining the significance ratio. A significance ratio of three or above is usually interpreted as constituting a significant change not due to chance, a ratio between two and three as being of doubtful significance, and one below two as more likely being insignificant. In addition, the coefficient of variation was computed for each organ series.

OBSERVATIONS AND DISCUSSION

Growth in Body Weight

A difference in constitution, especially that due to obesity, between normal and gonadectomized animals has long been known and was probably the first change noticed as a result of that operation. However, there is anything but uniformity in the changes observed as will be seen by studying the results published by Hatai (1913), Stotzenburg (1913), Cramer and Marshall (1923), van Wagenen (1928), Slonaker (1930), and Commins (1932). Korenchevsky (1925) states, "that there are 'fat' and 'thin' castrated animals and human beings. Metabolism was found to be lowered in 'fat' castrated animals, but not in 'thin' castrated ones." This dual response seems for the most part to be responsible for the contradictory results reported, at least for the more carefully performed

experiments with rats. This inconsistency in response is also evident from the much higher coefficients of variation in the spayed for the weights of the body, head, and integument (Table 1).

TABLE I

ORGAN WEIGHTS, SIGNIFICANCE RATIOS AND COEFFICIENTS OF VARIATION

	Mean Spayed	Mean Controls	Significance Ratio	Coefficients of Variation	
				Spayed	Controls
ody weight	249.28	212.68	6.5	15.01	8.55
ody length	20.50	20.40	.9	3.12	2.82
ail length	20.22	19.77	2.7	4.58	3.96
ead	20.2	19.0	4.0	9.34	6.10
ntegument	39.0	31.2	7.4	17.68	9.70
umerus weight	.3050	.2927	3.3	7.20	5.96
umerus length	2.57	2.56	.5	2.26	1.97
emur weight	.6766	.6584	1.7	9.06	7.40
emur length	3.37	3.35	1.1	2.48	2.35
ypophysis	.0117	.0116	.5	12.27	14.25
hyroid	.0197	.0208	1.4	18.74	19.52
uprarenal glands	.0336	.0508	12.7	15.24	16.90
hymus	.4784	.2935	9.4	27.76	20.24
rain	1.8255	1.8457	1.3	5.04	3.96
pinal cord	.5698	.5819	1.6	6.53	7.35
yeballs	.2809	.2806	.2	3.87	3.37
eart	.8951	.8503	2.7	10.99	8.99
ungs	1.8080	1.5705	2.6	31.40	23.61
ubmaxillary glands	.4482	.4360	1.2	13.37	10.61
limentary group	18.504	17.296	2.4	12.91	17.16
tomach	1.0104	.9281	4.6	8.35	10.89
ntestines	4.0103	3.9782	.3	11.38	13.32
iver	7.7553	8.1107	1.9	12.68	12.10
pleen	.5084	.4800	2.1	15.78	11.94
idneys	1.6558	1.7094	1.9	9.65	7.52
terus	.0337	.5634	23.5	15.94	29.64

In our series (Figures 1-3, Table 1) the gain in body weight amounted to 26 per cent for the first group, and to 7 per cent for the second group, resulting in a total increase of 17 per cent of the spayed over the controls. The significance ratio for this difference was 6.5. This closely resembles

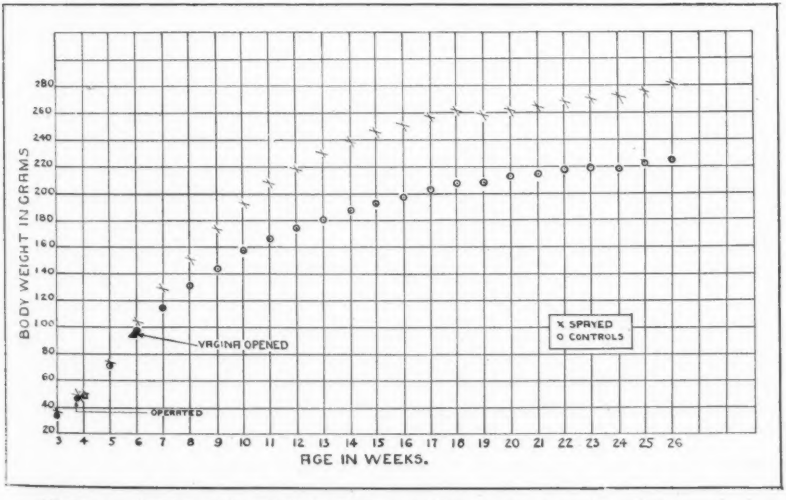

Figure 1. Average body weight in grams according to age in weeks. First group.

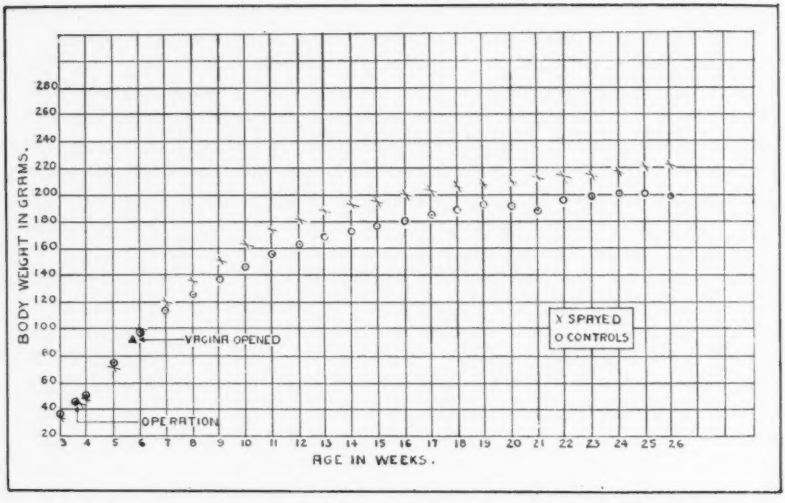

Figure 2. Average body weight in grams according to age in weeks. Second group.

the two series of Stotzenburg and Hatai. Hatai states that his series was not as obese and in a more emaciated condition than the series of Stotzenburg. This difference seems to be present in our series. In a few cases a severe diarrhea, which appeared in the colony during that summer, was responsible for the emaciated condition. Furthermore, the spayed rats of the second series did not grow to be quite as heavy as the controls of the

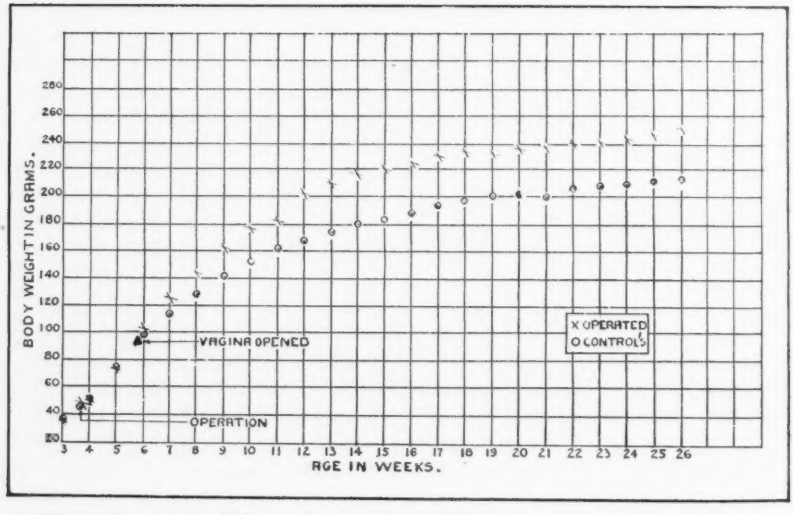

Figure 3. Average body weight in grams according to age in weeks. Both groups combined.

first series. It is evident, therefore, that the unfavorable conditions prevailing during the summer months suppressed the factors responsible for the overgrowth in the spayed much more than in the normal body growth of the controls. Further work will be required to work out such a seasonal difference, as seems to exist, not only as regards body growth, but also with respect to other organs. Considering the group as a whole, it was observed that the fat deposit was greater in the spayed than in the controls. Doubtless, the difference in body weight between the spayed and the controls is in part due to the greater deposition of fat in the spayed.

From the growth curves (Figures 1-3) it is seen that both the spayed and the controls grew equally well up to the time of sexual maturity, as manifested by the opening of the vagina in the controls. After that, the spayed diverge with ever-increasing increment, the curves tending to run parallel toward the end of the observation period. A slight retardation in growth occurred for a few days following the operation. This was the case in both spayed and controls.

In close relationship with the overgrowth and obesity of the spayed rats are the findings of McKeowen and associates (1933), namely, that the spayed rats eat more, and of Korenchevsky (1925), that metabolism is diminished in those spayed animals which show an abnormal accumulation of fat. Slonaker (1930) found the intake, measured in calories, to be less in the spayed. However, computing the energy expenditure in calories, he showed that the spayed had a much higher metabolic surplus, some of which was probably stored as fat.

Body Length

In the first group of our series, there was a slight increase in body length in the spayed (one per cent), but none in the second group. Taking the entire group (Table 1), a significance ratio of 0.9 was found for the difference in body length. The difference cannot be regarded as significant.

Tail Length

Both of our groups (Table 1) showed an increase in tail length in the spayed, and since the significance ratio for this difference is 2.7 it must be regarded as probably significant. The difficulty encountered in measuring tail and body lengths accurately may be responsible for some of the discrepancies in the results of various investigators. Representing, as they do, changes in the axial skeleton, further comment regarding these measurements will be made in discussing the skeletal system.

Age at Which the Vagina Opens

The vagina failed to open in all the spayed rats of the entire group. In some instances only there was a pin-point opening, not any way comparable to the normal physiologic destruction of that membrane in the controls, which occurred normally at an average age of 41 days, and average weight of 94 grams.

In one rat, not included in our series, in which a small piece of one ovary had been left in place, the vagina opened after 40 days, the rat then weighing 84 grams. In other respects this animal seemed to be intermediate in its changes. This was especially evident in the size of the uterus which, weighing 0.340 grams, was about ten times as heavy as the mean weight of the uterus in the spayed and about one-half of the mean weight of this organ in the controls.

Occurrence of Infections

Infection in the nasal and middle ear cavities was found to be equally common in the spayed and in the controls. The occurrence was much higher in the second group, another factor responsible for their poorer growth. On the other hand, the incidence of lung infection was higher in the spayed, again occurring chiefly in the second group (see below). Slonaker found the longevity of the spayed rats to be diminished; and since both normals and spayed died as a result of lung infection, it is possible that the earlier submission of the spayed rats to this infection is responsible for their shorter span of life.

Weight and Length of Individual Organs

Head. The head (Table 1) was significantly heavier in the spayed than in the control rats, the significance ratio being 4.0. This increase in weight may be due to an increase in the weight of the skull, or of the soft parts, or due to both. Certainly, the integument was responsible for part of the increase.

Integument. The weight of the integument (Table 1) was very markedly increased in the spayed. The significance ratio of the difference was 7.4. Much, if not all, of the increase was most likely due to the deposition of fat.

Skeletal System. In our group (Table 1) no significant changes were found in the length of the humerus and femur, and, as previously mentioned, in body length. No significant change in the weight of the femur was found, but the humerus was heavier in the spayed, the significance ratio being 3.3. There seems to be no good reason why there should be no difference in the lengths of the body, humerus, femur, and weight of femur, but an increase in tail length and in the weight of the humerus. One is inclined, therefore, to think of some experimental error. At least, in view of these results, and in view of the already mentioned experimental errors, to which the earlier workers were so often subject, it seems advisable to use the utmost caution in conclusions as to any changes in the skeleton due to spaying.

Hypophysis. No change was found in the weight of the hypophysis (Table 1), the mean weight of the controls and the spayed being almost identical. The significance ratio for the difference was 0.5. The two groups were very much alike in their behavior. This, therefore, does not substantiate the findings of Hatai, and later emphasized by Livingston, that

little or no overgrowth in body weight is associated with a hypertrophy of the hypophysis. Certainly, if that were always the case, our second group would show more hypertrophy, there being so little increase in body weight as compared with the first group.

Thyroid. There was no significant change in the weight of the thyroid (Table 1). The significance ratio of the difference in weights was only 1.4, the gland weighing more in the controls.

Suprarenal Glands. There is a marked atrophy in the weight of the suprarenal glands of spayed rats. In our series (Table 1) we found a difference in weight of the suprarenal glands between the spayed and controls of 51 per cent. The significance ratio was 12.7. The difference in the first group was 61 per cent, and 44 per cent in the second. Data from an experiment of ours, not included here, seem to indicate that the atrophy of the suprarenals, as evidenced by gross weight, is only slight 60 days after operation, the operation having been performed, as in this series, when the rats were between 20 and 30 days of age.

The great atrophy in the suprerenal glands due to spaying is even more interesting in the light of the recent report by Leiby (1933) of a 112.5 per cent increase in the weight of rat suprarenals due to injections of theelol, one of the ovarian hormones.

Thymus. The thymus (Table 1) was markedly larger in the spayed animals, the significance ratio being 9.4.

Brain. No significant change was obtained in the brain weight (Table 1), the significance ratio being 1.3. In both groups there was a slight decrease in weight in the spayed animals.

Spinal Cord. Results similar to those for the brain weight were obtained for the weight of the spinal cord (Table 1). The significance ratio, 1.6, was slightly higher. Both of our groups showed a slight decrease in weight for the spayed.

Eyeballs. The mean weight of the eyeballs of the spayed animals was practically the same as that of the controls (Table 1), the significance ratio being 0.2. This absence of any difference occurred in both groups.

Heart. The heart (Table 1) in our spayed rats weighed more than in the controls. With the general overgrowth of the spayed, it seems reasonably possible for a compensatory hypertrophy to take place. It seems likely, therefore, that our significance ratio of 2.7, while not absolutely significant, may be interpreted as probably significant.

Lungs. A similar compensatory hypertrophy, as suggested for the heart, might be expected for the lungs. We find then, too, a significance ratio of almost equal magnitude, amounting to 2.6, the lungs (Table 1) weighing more in the spayed group. However, the fact that part of the increase in the weight of the lungs of the spayed animals is due to disease may be seen from a comparison of the incidence of lung infection. Eight spayed rats showed smaller areas of consolidation, and 2 others had exten-

sive lesions, as compared with 3 and 1 for the controls, respectively. This has produced a much wider variability, as is expressed by the coefficients of variation, amounting to 31.4 for the spayed, and 24.6 for the controls.

Submaxillary Glands. There occurred an increase in weight in the spayed in the first group, but a slight decrease in the second group, resulting in a slight increase in the spayed when the whole group is considered, the significance ratio being 1.2 (Table 1).

Alimentary Group. The alimentary group of the spayed animals in the first group showed a marked increase in weight, but there was a slight decrease in the second group. Nevertheless, the significance ratio was 2.4, the alimentary group being heavier in the spayed animals (Table 1). This increase was partly due to an increase in the weight of the stomach, and possibly to deposition of fat in the mesenteries, and differences in amount of contents in the stomach and intestines. The difference between the two groups is probably in relationship with the marked general overgrowth of the first group as compared with the relatively slight changes of the second group in this respect. A somewhat greater variability is found in the controls with a coefficient of variation of 17.2 as compared with 12.9 for the spayed. Both the stomach and intestines help to make this difference.

Stomach. Since it was observed that spayed rats eat more (McKeowen, et al., 1926), it was interesting to note whether this would be manifested by a compensatory hypertrophy. A marked increase in weight (Table 1) was actually found in both groups, the significance ratio being 4.6.

Intestines. Practically no difference in weight was obtained in this organ (Table 1), as is also expressed by the significance ratio of 0.3.

Liver. Both groups (Table 1) showed a decrease in weight of the liver in the spayed animals. The difference does not seem entirely negligible; yet, with a significance ratio of 1.9 one is not justified in accepting any significance.

Spleen. An indefinite change was found in this organ (Table 1), similar to that found in the liver. The change, however, was in the opposite direction, and was slightly more significant. The significance ratio was 2.1. The coefficient of variation was also somewhat larger in the spayed, being 15.8 for the spayed as compared with 11.9 for the controls.

Kidneys. Changes similar in every respect to those found for the liver were obtained for this organ (Table 1), there being in both groups a decrease in weight in the spayed. The difference was much greater in the second group. The significance ratio for the difference was 1.9.

Uterus. The marked atrophy in the uterus (Table 1) in the spayed animals, although expected, was striking as to degree. The uterus weighed 17 times as much in the controls as in the spayed. In the controls the coefficient of variation, 29.6, was much higher than in the spayed group, in which the coefficient was 15.9. This was probably due to oestral changes in the controls, these cyclic changes being absent in the spayed.

SUMMARY AND CONCLUSIONS

In total 50 rats were used: 25 were spayed and 25 were used for controls. They were operated upon at an average age of 26 days, when they weighed about 47 grams. The ovaries were removed through an abdominal incision. Ether anesthesia was used. All animals were killed and completely autopsied at six months of age.

The spayed and controls grew equally well up to the time of sexual maturity, as manifested by the opening of the vagina in the controls at 41 days. The weight at this time was about 94 grams. Thereafter the spayed group grew much more rapidly, weighing 17 per cent more at the time of autopsy. Part of this weight increase occurred in the head which was significantly heavier in the spayed animals. The integument was also much heavier, and there was a greater accumulation of fat in the spayed animals. Body length was not altered, but the tail was a little longer (probably insignificantly) in the spayed group. In the spayed animals the vagina did not open.

No change was found in the length of the femur and humerus or in the weight of the femur. The humerus was found to be heavier in the spayed. No change in weight was found in the hypophysis and the thyroid. In the spayed there was a marked atrophy in the suprarenal glands and a much larger thymus.

No significant changes were found in the weights of the brain, spinal cord, and eyeballs.

The heart and lungs were enlarged (differences probably significant) in the spayed. Some of the increase in the weight of the lungs is due to a greater incidence of infection in the spayed.

No significant differences were found in the weights of the submaxillary glands and the intestine. The alimentary group was heavier (probably significantly) in the spayed and the stomach was also significantly heavier. The liver and kidneys showed a tendency to be lighter, and the spleen to be heavier, in the spayed. These changes, however, are of doubtful significance. The uterus in the spayed animals was found to be one-seventeenth of the normal size.

REFERENCES

Anderson, D. H. and H. S. Kennedy: J. Physiol. **79**: 1933.
Commins, W. D.: J. Exper. Zool. **63**: 573. 1932.
Cramer, W. and F. Marshall. Quoted by Mottram and Cramer: Quart. J. Exper. Physiol. **13**: 218. 1923.
Hatai, S.: J. Exper, Zool. **15**: 297. 1913.
————.: J. Exper. Zool. **18**: 1. 1915.
Jackson, C. M.: J. Nutrition, **3**: 61. 1930.
Korenchevsky, V.: Brit. J. Exper. Path. **6**: 21. 1925.
Leiby, George M.: Proc. Soc. Exper. Biol. & Med. **31**: 15. 1933.
Livingston, A. E.: Am. J. Physiol. **40**: 153. 1916.
Marassini, A. and L. Luciani. Arch. Ital de biol. **56**: 395. 1911.
McKeowen, R. M., S. C. Harvey and R. W. Lumsden: Arch. Surg. **26**: 430. 1933.
Slonaker, L. R.: Am. J. Phyiol. **93**: 307. 1930.
Stotzenburg,, J. M.: Anat. Rec. **7**: 183. 1913.
van Wagenen, G.: Am. J. Physiol. **84**: 461. 1928.

Book Reviews

THE ADRENAL CORTEX: A SURGICAL AND PATHOLOGICAL STUDY. Broster, L. R. and H. W. C. Vines. H. K. Lewis & Co., Ltd., London. 1933.

The authors have made special study of the adreno-genital syndrome. They have confirmed previous observations that some adrenal tumors produce virilism and others do not. Ten cases are reported in which unilateral adrenal extirpation was made in the hope of ameliorating the adreno-genital syndrome in women. All the patients made complete recoveries and the general health is stated to have been in no way affected. One patient, however, at the time of report was bedridden and suffering from "great muscular weakness and lack of energy." A differential stain (Ponceau Fuchsin) was applied to the glands removed at operation and each was found to take a bright red color. Adrenals from normal subjects were found to stain lightly or not at all with this dye. In some of the cases in which virilism was manifest the adrenal showed no hypertrophy but gave the positive staining reaction. A similar positive reaction was obtained in the cortex of an adrenal removed for what was tentatively considered a case of Cushing's syndrome. In several instances the adrenalectomy was followed by considerable improvement in the adreno-genital syndrome. Six additional cases are reported in which laparotomy failed to show evidence of adrenal involvement, hence adrenalectomy was not performed. Two cases are reported of apparent virilism in women but in whom operation disclosed the presence of testes, hence they were diagnosed as of pseudohermaphroditism. Histological studies were made of the adrenals in autopsy material, including eight cases of cortical adenomata not associated with virilism, eight cases of cortical carcinoma, and sixty-five foetuses of various ages. The theoretical aspects of the problem received considerable attention. It is concluded that adolescent virilism of adrenal origin may occur with or without cortical hyperplasia; where hyperplasia is present, it is usually of simple type, malignant hyperplasia with virilism occurring usually in children and in adults. Simple or malignant hyperplasia of the adrenal cortex occurs more commonly without virilism than associated with it. In adolescent virilism a fuchsinophil substance was present in the cytoplasm of the cortical cells in considerable amount. In a small series of normal fresh adrenals it was present in traces or not at all. In adrenal carcinomata associated with masculinization, a similar substance was found in the carcinoma cells, but was absent in growths which were not accompanied by sexual changes. A substance showing a similar staining reaction was seen in the cortical cells of the male foetus between the foetal ages of ten and seventeen weeks. A similar substance was seen in the cortical cells of the female foetus for a much shorter

period, between the foetal ages of eleven and fifteen weeks. In both sexes this substance was found only in traces during the latter part of intrauterine life. A similar substance was seen in the cortical cells in a case of hermaphroditism. In two female foetuses the persistence of the staining reaction was abnormal both in amount and in time, and was accompanied by a hyperplasia of the anterior pituitary. It is inferred that masculinization of adrenal original is related directly to the normal "male phase" of the female cortex at the fourteenth week of development. It is suggested that hermaphroditism may be the immediate and virilism the delayed or latent manifestation of a failure in the normal control of cortical activity at this period. It is suggested that the masculinization hormone of certain adrenal carcinomata is referable also to the same period in the development of the female adrenal.

THE CHEMISTRY OF THE HORMONES. Harrow, Benjamin and Carl P. Sherwin. The Williams and Wilkins Company, Baltimore. 1934.

As stated by the authors, the attempt was to write a practical book for use of laboratory workers who wish to prepare active hormone fractions or to isolate chemically pure hormones; and of use to the student who wants a connected account dealing with the chemical characteristics of the hormones insofar as they are at present known. In addition to their own well-known technical competence the authors have brought to bear also the knowledge of several able students who read various chapters prior to publication. These included Drs. Collip, Doisy, Foster, Hisaw, Ivy, Jensen and Swingle. The result is a book that can be highly commended to students in this field. Not the least valuable feature of the books is an excellently selected bibliography at the close of each chapter.

The book is largely free from statements to which exception can be taken. Some question can be raised as to the judgment expressed of relative importance of contributions and rarely of priority. But in a field in process of so rapid development as endocrinology complete omniscience is available to no one. It seems to the reviewer that Hanson's work on parathyroid hormone receives something less than its due attention. Also the important contributions of Zuelzer to the solution of the insulin problem deserve attention. On the other hand, the difficult section on the adrenal cortex hormones has been admirably treated. A somewhat remarkable exception to the general tone of judicious conservatism of the book is the extravagant statement that the number of alleged hormones in the pituitary is legion and their number is augmented daily.

The work represents on the whole an excellent piece of book-making, creditable alike to the authors and the publisher. It can be highly recommended for the necessarily brief period during which it will be up-to-date and will be of permanent value, as far as fundamentals are concerned, for years to come.

GLANDES ENDOCRINES. MÉTABOLISME DE L'EAU ET DIURÈSE; LES OEDÈMES ENDOCRINIENS. Langeron, L., M. Paget and J. Ledieu. Pp. 276. G. Doin & Cie, Paris. 1934.

Following a brief review of water metabolism in general the clinical and certain experimental aspects of the relationship between the endocrine glands on the one hand and the composition of the blood, diuresis and oedema on the other, are discussed in considerable detail. The first part of the book composed of eleven chapters is devoted to the clinical side of the problem. The authors discuss some endocrine types of oedema and elaborate the subject of opotherapy in these conditions as well as lipoid nephrosis, eclampsia, angioneurotic oedema, liver disease and oedema of non-endocrine pathogenesis.

The second section of four chapters considers the literature bearing on the experimental and biochemical aspects of the subject.

The last section of two chapters is concerned with the authors' personal experimental and biochemical studies. They supplement current ideas, notably in the response obtained from thyroid extract. They report that when the ration of plasma volume to cell volume is less than 1.35 repeated doses of thyroid extract produce at once a passage of water, chlorides and protein from the tissues or organs into the blood. They have coined the term "plasmhemiasis" for this phenomenon. The plasmhemiasis is followed later and secondarily by an increase in diuresis under thyroid. The plasmhemiasis is less notable in the cases in which the blood is originally rich in liquid constituents.

The effects of single as well as repeated doses of endocrine medicaments, at times in combination with diuretics, were investigated. Many of the experiments were too few in number to make final conclusions. The need for further research is stated.

The first two sections offer a fairly comprehensive review of the literature although somewhat weak in American references which would have proved helpful in eliminating some of their experimental problems. The book is very well organized and well written (aside from frequent mistakes in the spelling of proper names) and will prove useful particularly to clinicians.

Abstract Department

The adrenals and glucose tolerance. Banerji, H. and C. Reid, J. Physiol. **81:** 93. 1934.

The method employed utilized two series of albino Himalayan rabbits. Group 1 consisted of bilaterally adrenalectomized animals and was further divided into sub-groups: (A) those animals which died in a short time post-operatively with signs of grave adrenal insufficiency; (B) those animals which survived the operation for a long time without any such signs. Group 2 consisted of animals in which the medulla was destroyed but the cortex was left uninjured in so far as possible. Intravenous injection of glucose (1 g./100 cc. blood) was followed by blood sugar determinations at 15, 60, and 90 minutes. Fasting blood sugar in animals with symptoms of adrenal insufficiency was lower than normal, slightly lower in the remaining groups of operated rabbits. Injected glucose disappeared rapidly from the blood of both normal and operated animals. In animals with signs of cortex insufficiency, glucose disappeared more rapidly from the blood in the course of 15 minutes. This increased rate of disappearance was not maintained. The glucose tolerance of rabbits surviving adrenalectomy for a long time and those in which the medullae alone were removed did not differ significantly from normals. The authors conclude that the tissue sugar of animals showing cortex insufficiency is low, but that the tissues have retained their ability to remove injected glucose from the circulating blood.—G. W. Thorn.

The apparent prepotent function of the glands. Britton, S. W. and H. Silvette, Am. J. Physiol. **100:** 701. 1932.

Studies were made on liver and muscle glycogen, blood sugar and lactic acid values, in untreated, extract-treated, adrenalin-injected, and glucose-injected adrenalectomized cats. Unoperated animals served as controls. Two groups of experiments were also made with adrenalectomized rats (11 animals). In the untreated adrenalectomized series blood glucose, liver glycogen, and muscle glycogen showed marked reduction from normal levels, while blood lactates increased. The glycogen of heart muscle was not reduced. Such changes are not observed after extirpation of the adrenal medulla alone. The animals also showed a markedly reduced ability to store liver glycogen under heavy glucose dosage; normal animals stored 8 to 10 times as much. After pancreatectomy or hepatectomy (functional) carbohydrate metabolism was no more severely affected than in adrenal insufficiency. Administration of cortico-adrenal extract brought about recovery of animals in the terminal stages of insufficiency, the recovery being accompanied by increments in blood sugar to normal or even to hyperglycemic levels, increases in liver and muscle glycogen, and diminution of blood lactic acid. Cortico-adrenal extract also increased the blood sugar and the liver and muscle glycogen in normal, young animals. Control experiments showed that the results were not produced by the small amount of adrenalin (1:2,000,000) contained in the extract. It was concluded that the cortico-adrenal tissues and their hormone as contained in the extracts are primarily concerned in the maintenance of normal glucose and glycogen levels in the body, and that regulation of carbohydrate metabolism may be considered as the prepotent function of the adrenal glands.—L. C. W.

The mechanism of the nervous discharge of adrenaline. Feldberg, W., B. Minz and H. Tsudzimura, J. Physiol. **81:** 286. 1934.

In eviscerated cats, especially after injections of eserine, stimulation of the splanchnics may produce falls of blood pressure and salivary secretion (not abolished by ergotoxine). These effects are suppressed by atropine. They are interpreted as due to acetylcholine liberated in the adrenal glands by the nerve impulses. The adrenaline discharge evoked by injections of acetylcholine or by splanchnic stimulation was found to be enhanced by eserine. It is concluded that acetylcholine is the humoral transmitter of splanchnic nerve impulses to the suprarenal medulla.—A. Rosenbleuth.

Studies on the suprarenal cortex; plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. Harrop, G. A., L. J. Soffer, R. Ellsworth and J. Trescher, J. Exper. Med. **58:** 17. 1933.

The concentrations of plasma electrolytes were studied in dogs suffering from adrenal insufficiency, and during recovery. In arterial blood plasma, the progress of insufficiency was marked by a fall in total base, due to the fall in Na, and a decrease in bicarbonate and chlorides which together were about equal to the decrease in Na. Mg and K rose, while Ca showed no marked change. Studies of water and sodium balance showed that these substances are lost through the urine. When cortico-adrenal extract is withdrawn, there is a great increase in Na excretion, and a strongly negative Na balance. As the suprarenal insufficiency progresses, the excretion of Na falls, due, the authors believe, to the depletion of the body stores, but even with the decreased excretion there is still a negative balance on account of the decreased intake. The loss of Na and Cl through the urine is greater than can be accounted for by fall in plasma concentration, so the excess must come from the water depots outside the plasma. When extract is given and the animal begins to recover from the insufficiency, there is retention of Na and Cl until the original condition is restored. The kidney is believed to be the focus of this regulatory function. The salt content of the food has a bearing on the cortico-adrenal extract requirement of the animal; on a high salt diet the dosage may be reduced to a fraction of that necessary on a low salt diet.—C. A. Winter.

The relation of bilateral suprarenalectomy and subsequent extract therapy on the body weight and oestrual cycle of the albino rat. Kroc, R. L. and S. J. Martin, Am. J. Physiol. **108:** 438. 1934.

Complete suprarenalectomy in 68 female albino rats resulted in the death of 93.0% of the group. The survival period ranged from 6 to 29 days. Definite accessory cortical tissue was found in those animals that survived for extended periods. Removal of both suprarenals at one operation shortened the survival period. This was three days less than the average of 14.9 days for rats whose suprarenals were removed in two stages. Following complete suprarenalectomy there was a gradual decline in body weight which at the death of the animal ranged from 13 to 35%. The average weight loss of a two-stage operated series was 21% as compared to 17% for a one-stage series. Definite oestrous disturbance occurred in 92.5% of the animals following removal of both suprarenals. No correlation was found between the degree of body weight loss, the survival period or pre-operative weight of suprarenalectomized female rats. Potent and standardized extracts of beef and horse suprarenals prepared according to the Swingle-Pfiffner method have successfully maintained the life and restored the normal oestrous cycle of suprarenalectomized rats. The cycles did not reappear until the body weight returned to approximately the pre-operative level. Our results favor the theory that the active principle from the suprarenal cortex is a general tissue hormone. It is concluded that the extracts employed in this work contained no ovary-inhibiting or specific sex-stimulating factor.—Authors' Summary.

Biological assay of cortico-adrenal hormone by means of a morphine resistance test (Titulacion de la hormona cortico-suprarrenal por la resistencia a la morfina). Leloir, L. F. and A. Novelli, Rev. Soc. argent. de biol. **9:** 273. 1933.

The method proposed by Lewis and de Meio has been subjected to further study and the following procedure, based on morphine toxicity curves of adrenalectomized rats treated with varying doses of cortico-adrenal extract, is advised. The extract was injected during 3 days following adrenalectomy and then 8 mg. morphine hydrochlorid per 100 grams weight was injected subcutaneously. Groups of 10 rats should be used and mortality in 24 hours noted. The unit is considered as that amount which permits 30% survival.

—J. T. L.

Non-protein blood sulphur and the adrenal glands (El S no proteico sanguíneo y su relación con las suprarrenales). de Meio, R. H., Rev. Soc. argent. de biol. **9:** 325. 1933.

Non-protein blood surphur was determined in dogs by the author's method. Unilateral adrenalectomy does not modify the concentration of total sulphur in the blood; in 4 of 8 experiments an increase in neutral sulphur with a cor-

responding decrease in sulphates was observed. Following complete adrenalectomy after a short delay (±24 hour) total sulphur and sulphates increased progressively so that at death 3 and 5 times the initial value was seen. The neutral sulphur varied irregularly and in 5 of 8 experiments an increase was seen just before death. Comparing the values for total sulphur with those for non-protein nitrogen it is deduced that most, if not all, the increase is due to retention owing to impaired kidney function.—J. T. L.

Potential adrenaline (L'adrénaline virtuelle). Mouriquand, G., A. Leulier and L. Revol. Rev. franç., d' endocrinol. **11:** 324. 1933.

The authors review their experiments and the confirmatory work by others in support of their hypothesis that there exists in the fresh suprarenal gland, in addition to free adrenaline, a certain amount of "potential" adrenaline which is not detectable by chemical or physiological reactions for adrenaline until after the lapse of a certain amount of time with proper preservation. Free and potential adrenaline both appear to be localized in the medullary zone of the suprarenal, although one can sometimes find very small amounts in the cortical zone. Certain toxins, e.g., tetanus toxin, affect the adrenaline content of the adrenals slightly or not at all; but, on the contrary, inanition, botulinus toxin, and, above all, diphtheria toxin, empty the suprarenals of their adrenaline content. Adrenaline disappears rapidly after death. The last two facts are thought to be of practical importance, since in using suprarenal glands for opotherapy it is important, therefore, to use glands fresh from healthy animals.—C. C. Franseen.

Adrenal sexual precocity; caused by tumors of adrenal cortex; case report of boy 5 years of age. Player, L. P. and H. Lisser, Urol. & Cutan. Rev. **37:** 758. 1933. Abst., J. Nerv. & Ment. Dis. **79:** 691.

This paper records the case of a boy 4 years and 11 months old whose bone age was that of 12 or 13 years. He was tall and large, though not obese. The external genitals were adult. He had the beginnings of a moustache, axillary hair, and pubic hair. The prostate was of almost adult size, and its secretion contained much lecithin and many spermatozoa. He had frequent erections and many nightly emissions. Pyelography showed a round tumor above the left kidney. A diagnosis of left adrenal cortical tumor was made and confirmed by operation, when a well-encapsulated mass the size and shape of a large apple was removed. Histologically this was composed of rapidly growing adrenal cortical cells. When seen 20 months after the operation the boy was in good health, and showed the following changes of endocrine abnormalities. The axillary hair had vanished, the moustache and pubic hair had thinned somewhat, the boy was more childish in appearance and demeanor and though the external genitalia had not diminished in size erections and emissions had been rare since the operation. The writers report eight other instances of sexual precocity in boys in which an adrenal cortical tumor was found at necropsy. In three of these operation was attempted, but the tumors were inoperable in two. In the other case the tumor was removed, but death took place 12 hours after the operation.

The adrenal cortex and sex; the influence of cortical extract on normal and castrated males. Simpson, S. L., A. Kohn-Speyer and V. Korenchevsky, Lancet, **2:** 1194. 1933.

Effects of the injection of adrenal cortical extract on the growth and development of the reproductive system and other organs of immature normal and castrate male rats were studied. The extract was made by the Swingle-Pfiffner method, and 3 cc. per day were necessary to maintain the life of an adrenalectomized cat. Five litters, averaging 5 each, of normal rats were treated with extract dosages varying from 0.2 cc. to 1.2 cc. per day. Injections were begun at 22 or 23 days and continued for 14, 21, and 28 days. Two other litters were castrated at 26 days of age and 6 days later injections were begun and carried on for 29 days in one litter, for 42 days in the other. The treatment failed to cause any change of weight, as compared to controls, of the prostate, seminal vesicles, penis, testis, adrenals, thyroid, pituitary, thymus, liver, kidneys, spleen, and heart; and no change in fat deposition. Microscopic examination of the testes showed no departure from the normal picture as a result of the treatment. Castrate involution was not prevented. In an attempt to reconcile these negative results with the clinical evidence indicating the ex-

istence of an adrenal-gonad relationship three possibilities are discussed: (1) that there might be an undetected basophilic adenoma of the hypophysis associated with tumors of the adrenal cortex; (2) that the hypernephroma cells are really primitive sex cells located in the adrenal cortex as a result of the related embryonic origin of the gonads and the cortex; (3) that the extracts used contained the life-maintaining but not a sex-stimulating factor.—R. Gaunt.

The relation between blood pressure, blood urea nitrogen and fluid balance of the adrenalectomized dog. Swingle, W. W., J. J. Pfiffner, H. M. Vars and W. M. Parkins, Am. J. Physiol. **108:** 428. 1934.

Twenty adrenalectomized dogs were studied with regard to the relation of the blood urea nitrogen and the blood pressure. An inverse relation exists between the two throughout the cycle of insufficiency and recovery on hormone treatment. The elevation of the urea nitrogen occurs simultaneously with the fall in arterial pressure and does not precede it. The azotemia of adrenal insufficiency is probably extra renal in origin and may have no necessary relation to the kidney. The phenomenon is not peculiar to adrenal insufficiency but is common to those other morbid states where dehydration and base and chloride deficit occur. It appears to have a significant (but ill understood) relation to the changed electrolyte pattern. The fall in arterial pressure in insufficiency is itself probably merely a reflection of the decline in volume of circulating fluid and associated electrolyte changes. The water balance of six adrenalectomized dogs was studied. The animals not receiving cortical hormone excrete daily by way of the kidney a greater volume of water in relation to the total fluid intake, than they do during the control period. The amount of fluid lost as urine (over total fluid intake) is inadequate to account for the dehydration and associated changes of adrenal insufficiency. A representative case, for example, lost but 430 cc. (over total fluid intake) during the entire insufficiency cycle. A much greater loss of fluid (over intake) occurs during the recovery phase of the cycle, following injections of cortical hormone (896 cc. as compared to 430 cc. in a representative case) than the animal loses during insufficiency. If the negative water balance of the animal in insufficiency is considered in conjunction with the fact that in such animals the mechanism for mobilization of fluid and salt, i.e., blood dilution, is lacking or greatly impaired, an adequate explanation is afforded for the observed phenomena. During the recovery phase, although the fluid loss is greater than during insufficiency, the fluids (and electrolytes) of the tissue and interstitial spaces are freely mobile.—Authors' Summary.

Effect of extract of the suprarenal cortex on maxillary sinusitis in the rabbit. Wenner, W. F., Arch. Otolaryng. **17:** 774. 1933. Abst., Am. J. M. Sc. **186:** 882.

The author studied the effect of extract of the suprarenal cortex on maxillary sinusitis, which he produced in 52 rabbits by irrigating the paranasal sinuses every 48 hours for 10 days with from 0.3 to 0.5% solution of sodium alizarin sulphonate. When a nasal discharge appeared, the rabbits were divided into two groups. One was given 3 intramuscular injections of the extract of suprarenal cortex in doses of 0.5 cc. per kilogram of body weight over a period of 1 week. The second or control group received no extract. Both control and experimental animals were killed 17 days after the last sinus irrigation, and the antral mucosa was removed. On microscopic examination the antral mucosae of the control rabbits showed a well-defined inflammatory reaction; whereas, no evidence of infection was noted in the antral linings of the animals receiving the suprarenal cortical extract, which produced a consistent decrease in hemoglobin, a change in alkali-reserve, and an increased number of vacuoles in the cytoplasm of the cells of the suprarenal cortex.

Endocrine studies; the gaseous metabolism of some dwarfs and giants. Rowe. A. W., J. Nutrition, **7:** 573. 1934.

During the visit of a circus troupe to Boston opportunity presented for a partial metabolic study of a group of dwarfs. For purposes of contrast and comparison an acromegalic giant, a "fat lady," and certain other members of the troupe were investigated and these records were supplemented in turn by the reports from a small group of patients presenting like conditions. The present report deals with the energy metabolism as measured by the oxygen exchange. As the large people require extrapolation and the small individuals raise a further question as to the proper basis for the estimation of normal

metabolism, analysis has been made of the several usual prediction standards and the applicability of each to the individual subjects in a series is discussed. Depressed basal rates were the rule; in each case, however, a summary of the results of comparison of the individual observed rate with the several prediction standards reflected the physical and functional status which had engendered the specific somatic abnormality. The extremes of height were respectively 95.0 and 228.6 cm.; of weight, 16.3 and 189.5 kg.; and of oxygen consumption per minute, from 76.6 to 419.7 cc. Abnormal pituitary function was a feature of a majority of the group.—Author's Abst.

Undescended testicles in man and rhesus monkeys treated with anterior pituitary-like principle from the urine of pregnancy. Aberle, S. B. D. and R. H. Jenkins, J. A. M. A. **103:** 314. 1934. Abst., A. M. A.

The authors found that injections of the anterior pituitary-like principle from the urine of pregnancy caused the testicles to descend in two of four boys. In only one instance was the descent complete. The substance caused hypertrophy of the scrotum and testicles and in one instance a growth of the penis. In immature monkeys (Macaca mulatta) with unilateral orchidectomy, this hormone caused complete descent of the testicle in one animal and partial descent of the testicles in four. In these four animals the fascia surrounding the vas deferens and spermatic vessels was too short to allow the testicles to reach the lower part of the scrotum. The failure of the treatment may have been due to an incorrect amount of hormone, to age, to nutrition or to some factor related to the activity of the other endocrine glands. In man a possible external mechanical obstruction and developmental irregularities must be considered. Microscopic examination of the testicles of the injected monkeys showed marked tubular enlargement, a corresponding increase in interstitial material, but no mature sperm. Identical amounts of the anterior pituitary-like factor caused various degrees of hypertrophy in the prostate, seminal vesicles and testicles of immature monkeys. The total dosage of the principle administered is not in direct proportion to the distance that the testicles descend either in man or in monkeys.

The sexual variation in carbohydrate metabolism; the comparative glycogen and fat content of the liver and muscles of rats and guinea pigs. Deuel, H. J., Jr., Margaret Gulick, C. F. Grunewald and C. H. Cutler, J. Biol. Chem. **104:** 519. 1934.

The liver glycogen was higher in fasting male rats killed 24, 48, and 72 hours after the administration of glucose than it was in females. An abrupt rise occurred in the glycogen level in the female rats in one case after 96 and in two groups after 120 hours, which brought this constituent to a higher level than in males. It is suggested that this may be in the nature of a premortal rise. The liver fat was higher in the female than in the male rats during the whole period of fasting. There were no differences in glycogen level between the sexes in rats killed without fasting after a high carbohydrate diet. The liver glycogen was found to be higher in female than in male guinea pigs after 48 hours of fasting. Possible explanations for this discrepancy are offered. However, difference, due to sex, in liver lipid similar to those found in rats were obtained with guinea pigs.—Authors' Summary.

The effect of intravenous administration of the pregnancy urine factor on the ovaries of rhesus monkeys. Engle, E. T., Am. J. Physiol. **108:** 528. 1934.

The anterior-pituitary-like principle of human pregnancy urine not only failed to cause follicular activation, but definitely inhibited the production of oestrin, as judged by loss of color of sex skin and oestrin privea uterine bleeding. The previous observations on this point were extended by experiments on 9 rhesus monkeys. In 5 animals intravenous dosages, ranging from 3,000 to 9,900 rat units in four days, were given. The inhibition of oestrin production was accompanied by hyalinization of smaller follicles, extensive cytolysis of the granulosa, and thecal thickening. These morphological changes are interpreted as being various phases in follicular atresia. The ovary of the monkey was not damaged. In 1 animal it was reactivated within the normal time by an active fraction of the anterior pituitary: in another, it entered a normal cycle after cessation of treatment. The intravenous administration of this active principle to adult monkeys, either in massive doses or in very small doses, failed to cause oestrin production.—Author's Summary.

Gonad-stimulating hormone in urine of a patient with a teratoma testis. Fluhmann, C. F. and P. E. Hoffmann, Proc. Soc. Exper. Biol. & Med. **31:** 1013. 1934.

It was found, on the basis of 3 biologic tests, that the gonad-stimulating hormone in the urine of a patient with a teratoma testis had the same characteristics as the substance occurring in the urine of pregnant women.
—Authors' Summary.

Gonad-stimulating hormone in urine of a patient with a chorioepithelioma uteri. Fluhmann, C. F. and P. E. Hoffmann, Proc. Soc. Exper. Biol. & Med. **31:** 1014. 1934.

The gonad-stimulating hormone found in the urine of a patient with a chorioepithelioma uteri had certain biologic characteristics (stimulation of corpora lutea, lutein cysts, and follicles, and increasing weight of ovaries) similar to those of the substance occurring in women during normal gestation.
—M. O. L.

The sexual variation in carbohydrate metabolism; the effect of ovariectomy and theelin administration on the glycogen content of rats. Gulick, Margaret, L. T. Samuels and H. J. Deuel, Jr., J. Biol. Chem. **105:** 29. 1934.

The mean level of the liver glycogen for ovariectomized rats 48 hours after the administration of glucose in a dose of 5 mg. per sq. cm. of body surface was found to be 0.55%, while that of normal female animals following a like experimental routine equaled 0.25%. No variability in muscle glycogen was noted. Although the liver glycogen figures for the castrated females are higher than those found previously for normal males (0.45%), the difference is not great enough to be considered statistically significant. When theelin was administered to ovariectomized rats, a lower value in liver glycogen was obtained than that for the uninjected controls. However, inasmuch as the mean was considerably higher than that obtained for normal female rats in which the concentration of theelin probably is much less, it is not considered that the sex difference in carbohydrate metabolism previously found is solely dependent on the production of theelin.—Authors' Summary.

Investigation of the gonadotropic hormone in the gravid mare (Untersuchungen über die gonadotropen Hormone bei der graviden Stute). Hamburger, C., Endokrinologie, **13:** 305. 1934.

Hamburger performed experiments with the serum of pregnant mares tending to show that the gonadotropic hormone is derived in the case of the mare by the anterior pituitary lobe (and possibly also by the placenta). He used the serum or citrated plasma from 34 mares in the second to the fourth month of pregnancy and noted its effect on the ovaries of infantile mice. A definite stimulatory effect was obtained with only 1/200 cc. of serum indicating 200,000 mouse units per liter. The ovaries of the mice receiving varying doses of serum from mares, 60 to 74 days pregnant, were weighed and compared with the ovaries of non-injected mice and of mice receiving injections of gonadotropic hormone from pregnant urine. The serum was injected in doses of 1/80, 1/40, 1/20, 1/10, and 1/5 cc. The weight of the ovaries increased with the size of the dose until that of the mice receiving 1/5 cc. increased from 2 mg. to 10 mg. With the gonadotropic hormone from pregnant urine a plateau was reached at 4 to 5 mg. which did not rise with larger doses. One cc. of undiluted pregnant mare's serum (5 injections of 0.2 cc. each) injected into an infantile mouse resulted in ovaries weighing 35 mg. Further experiments were performed on 30 cockerels (400-500 gms. in weight). Ten received no treatment; 10 received daily injections for 7 days of pregnant mare's serum (60 mouse units per injection), while 10 were given daily injections for 7 days of a potent preparation (40 to 80 mouse units of prolan A and B) of the urine of pregnancy. The effect of the hormone on the growth of the comb was observed. At the end of one week the combs of the group receiving serum of pregnant mares were much larger and more hyperemic than those of the controls and those of the cockerels receiving the urine preparation. The testes of the control and urine-treated animals weighed on the average of 52 and 56 mg., while those of the serum-treated animals showed a gain of 268 mg. The author attributes the marked stimulation-effects of the pregnant mare's serum to the view that in the mare in the polyhormonal phase of pregnancy there follows an overproduction of "true" anterior pituitary hormone while the gonadotropic hormone in gravid women is produced by the placenta.—L. Goldstein.

Therapeutic value and effects of amniotin in gonorrheal vaginitis in children. Huberman, J. and H. H. Israeloff, J. A. M. A. **103:** 18. 1934. Abst., A. M. A.

The authors investigated the therapeutic value of amniotin in the treatment of gonorrheal vaginitis. The dosage consisted of 100 rat units of amniotin hypodermically three times a week to five patients, while one child, aged 3½ years, received daily from 120 to 200 rat units of amniotin orally. At no time were there any local or constitutional reactions manifested as a result of the treatment. In the three chronic cases the vaginal discharge disappeared after four weeks of treatment. The children of this group were clinically cured after receiving a total average of 21 injections or 2,100 rat units of amniotin. Vaginal smears taken weekly showed a progressive diminution in the number of intracellular diplococci and the appearance of an increasing number of cornified epithelium cells. The vaginal smears were negative for gonococci at the end of four weeks. The three acute cases required longer and more intensive treatment. The vaginal discharge disappeared after eight weeks of treatment. The children received a total average of 27 injections equivalent to 2,700 rat units. At the end of this period, vaginal smears were negative for gonococci. The child in this group who received amniotin by mouth required larger daily doses. However, the clinical results were similar to those of the other cases. Regardless of the amount of amniotin administered, the authors did not observe any hypertrophy of breasts or labia, or any uterine bleeding. Biopsies from the lateral wall of the vagina were made in each case before and after amniotin injections. The microscopic examination of the sections removed before treatment showed the thin delicate epithelial layers of the child's vagina, with marked round cell infiltration, significant of inflammatory changes. There was an absence of a definite cornified layer of epithelial cells. The examination of the biopsy sections after treatment revealed a definite increase in the number of epithelial layers, and the inflammatory changes were no longer present. The most significant change, however, was the presence of a definite layer of cornified epithelial cells. A direct relationship was observed between the clinical results and the histologic picture. The authors are of the opinion that the success of this mode of treatment depends mainly on the development of a layer of cornified epithelial cells. This layer of cells is analogous to the desquamating type of cells found in the normal adult vagina. The acquired layer of cornified epithelial cells acts as a protective barrier against the rapidly multiplying gonococci and thus prevents reinfections. On the other hand, the gonococci that have previously penetrated into the subepithelial spaces are destroyed by the normal phagocytic action of the leukocytic elements.

The Friedman rabbit ovulation test in differential obstetric diagnosis. King, A. G., J. Lab. & Clin. Med. **19:** 1033. 1934.

The results of 103 Friedman rabbit ovulation tests on 86 women are reported and discussed. The literature to date is reviewed. Of 4,500 cases, the gross error has been 3.9%; the corrected error, including the 26 false positive readings, amounts to 1.8%. The test is a valuable aid in differential diagnosis, provided its limitations are recognized. A positive test indicates usually, but not always, the presence of live chorionic tissue. It may become positive until pregnancy is well advanced, and if positive may not necessarily mean that the fetus is alive. The test should assist but not supplant the usual diagnostic technic.—Author's Summary.

A comparison of the Aschheim-Zondek and the Friedman tests in normal and abnormal pregnancy; an analysis of the literature and a report of the results obtained in 1112 cases. Mack, H. C. and G. H. Agnew, Am. J. Obst. & Gynec. **27:** 232. 1934.

An analysis of the literature on hormone tests for pregnancy and our own experience with 546 Aschheim-Zondek and 566 Friedman tests demonstrates a high degree of accuracy for both methods, the latter being slightly more accurate as well as easier and quicker. In proved cases of normal pregnancy an accuracy of 97.3% was obtained with the Aschheim-Zondek test and an accuracy of 97.8% with the Friedman method. In patients definitely determined not to have been pregnant an accuracy of 98.5% was obtained with both methods. In abnormal or interrupted pregnancy the result of the test should be interpreted with the clinical findings, a negative test signifies a nonpregnant state or an interrupted pregnancy, a positive test strongly indicates

living fetal elements, but due to a temporary persistence in elimination of the hormone, recent interruption or fetal death cannot be excluded. In cases of hydatidiform mole and malignant chorionepithelioma, the amount of hormone excreted is many times greater than that excreted during normal pregnancy. The persistence of positive tests after treatment of these neoplasms strongly suggests continued chorionic proliferation.—Authors' Summary.

Effects of castration in adult male. McCullagh, E. P. and J. F. Renshaw, J. A. M. A. **103:** 1140. 1934. Abst., A. M. A.

In order to increase familiarity with the diagnostic evidence of hypogonadism in men, the authors subjected 12 postpubertal castrates to careful clinical and laboratory studies, including bio-assays for the gonad-stimulating principles of the urine, and another substance from the urine, presumably a testicular hormone which promotes comb growth in capons. They present their observations, report a typical case and state that in a few such cases an attempt has been made at substitution therapy, with androtin, the male sex hormone prepared from urine. When sufficient material was available for adequate dosage, there was considerable improvement in the subjective symptoms of hypogonadism. These results have been most encouraging, but it is too early to evaluate accurately the benefits that may be anticipated from this type of treatment.

Studies in human physiology. VI. Variations in blood chemistry over long periods of time, including those characteristic of menstruation. Pucher, G. W., F. R. Griffith, Jr., K. A. Brownell, J. D. Klein and M. E. Carmer, J. Nutrition, **7:** 169. 1934.

Blood obtained from an arm vein under basal conditions in the morning, once a week, over periods of 1 and 2 years, from two men and two women was analyzed for urea, amino acid, uric acid, total creatinine, non-protein and undetermined nitrogen; sugar, cholesterol, chloride, calcium, inorganic phosphate and corpuscle volume. Statistical analysis of the data provides a measure of the constancy of blood composition and shows no correlation between concurrent variation of this and the basal metabolic rate. Menstrual variation: (1) Maximal during menstruation, declining uniformly to minimum toward end of intermenstrual period: chloride, uric acid and total creatinine; less certainly but probable, total non-protein, urea, amino acid and undetermined nitrogen. (2) Maximal about the time of menstruation but minimal midway between periods: calcium, cholesterol; doubtfully, sugar, inorganic phosphate. (3) Roughly the reciprocal of (2); blood oxygen and carbon dioxid capacity. Seasonal variation: (1) Minimal in winter or (for most) spring, maximal summerfall: oxygen capacity, cholesterol, inorganic phosphate, amino acid, total creatinine, and non-protein and possibly though less certainly, urea and undetermined nitrogen. (2) Reciprocal of (1), corpuscle volume. (3) Maximal in winter, minimal in summer; chloride, carbon dioxid capacity. The correlation of these with variation of other functions described in previous reports of this series is discussed.—Authors' Abst.

Biologic effects of hormone in lutein cysts in cases of hydatid moles and chorioepithelioma (Über biologische Auswirkingen von Hormonen in Luteincysten bei Blasenmole und Chorionepitheliom). Siegmund, H., Zentralbl. f. Gynäk. **58:** 1097. 1934.

Following curettement in cases of chorio-epithelioma or hydatid mole, large quantities of gonadotropic substance may continue to be secreted although the presence of chorio-epithelioma cannot be demonstrated histologically. The continued secretion of gonadotropic hormone might be attributed to the pituitary, although the possibility of a hormone reserve elsewhere in the body must be considered. The author cites four cases of hydatid mole or chorio-epithelioma in which the lutein cysts were rich in gonadotropic hormone (over 200,000 mouse units per liter of fluid in one case). In the case of lutein cysts the secretion of hormone becomes diminished from week to week whereas in the presence of chorionic tissue it is increased. Engorgement of the breasts is produced by the presence of lutein cysts. After the removal of the cysts, the breasts behave similarly to those after labor; the secretion of milk is due to the hormones contained in the ovaries. The gonadotropic substance stored in the lutein cysts in cases of hydatid mole or chorioepithelioma is secreted for weeks after the operative removal of the mole or tumor. In

this period of time it is hard to decide whether the gonadotropic substance is derived from the reserve in the cysts or if malignant growing chorionic villi remain.—L. Goldstein.

The response of the guinea pig mammary glands to injected sex hormones and ovarian grafts and its bearing on the problem of sex hormone antagonism. Smelser, G. K., Physiol. Zoöl. **6**: 396. 1933.

It was found that injections of oestrin in adult guinea pigs leads to just as marked growth of the nipples and mammary glands in the male as in the female. Oestrin alone, or injected along with effective doses of testis hormone, brings forth similar quantitative responses in the mammary glands of both sexes. Ovary grafts in normal males do not lead to mammary growth unless abdominal elevation of the testes or castration is performed. Ovarian transplants in castrated males seem to be periodic in function, as indicated by the responses of endometrial grafts in the anterior chamber of the eye, and by oestrin excretion. Ovarian grafts in experimental cryptorchid males reduce the intensity of testis hormone secretion since the oestrin secreted by the graft interferes with the activity of the pituitary.—H. Selye.

The effect on the weight of the offspring of administration of Antuitrin G to the pregnant rat. Sontag, L. W. and P. L. Munson, Am. J. Physiol. **108**: 593. 1934.

When Antuitrin G (Parke Davis) was administered to 12 pregnant rats in 1 cc. daily doses, the following results were noted: The number of still-births was increased about three times over the number in the control group of 16 pregnancies. The average weight of the 104 experimental pups was 0.4 gr. (7.3%) higher than that of the 149 controls. The period of gestation was increased in length in a majority of the litters but without a corresponding increase in birth weight of the pups over that of the remaining experimental litters. No significant correlation was found between age of mother and birth weight of pups, and a negative correlation existed between the birth weight of the pups and the size of the litter. A considerable variability in individual response to the Antuitrin G was found. The male pups showed greater weight increases than did the females. The weight advantage of 34 surviving experimental pups over 65 controls was much lessened by the end of the fifth day of postnatal life, but the experimental group maintained a slight superiority in weight through 50 days of age. The Antuitrin G injections did not incapacitate the rat mothers for subsequent reproduction.—Authors' Summary.

Polycystic ovaries in the newborn and early infancy and their relation to the structure of the endometrium. Spivack, Mary, Am. J. Obst. & Gynec. **27**: 157. 1934.

In a study of 36 cases, comprising fetuses, newborn, and infants, five of the newborns showed hyperplastic endometrium, an incidence of 14% for the whole group, and 22% for the newborn group, prematures and full term included. In no instance were there found distinct hypertrophic and hyperplastic uterine mucosae in the infants (one case of very mild stimulation was noted). Coexistence of polycystic ovaries and hyperplastic endometrium was observed twice in the newborn group only. Polycystic ovaries were found predominantly in infants, namely, in 85%, while in the newborn the incidence of this condition was 13%, or in the newborn at term 25%. Polycystic ovaries in infants of three weeks and older were not associated as a rule with hyperplastic and hypertrophic endometrium. There seems to be no causative relationship between cystic follicles of the ovaries and the structure of the endometrium in the newborn and in infants. In view of the greater predominance of polycystic ovaries in infants in comparison to the newborn, a suggestion is made that cystic degeneration of the follicles may be extrauterine in origin and may possibly depend upon the infant's own pituitary activity.—Author's Summary.

Note on ineffective use of theelin in a case of hemophilia. Tureen, L. I., Am. J. M. Sc. **188**: 216. 1934.

Previous reports indicate that an important relationship may exist between female sex hormone and hemophilia. Tureen attempted to render a hemophilic individual less susceptible to bleeding by the use of the theelin, injected intramuscularly, or administered by mouth, but without success. There oc-

curred no constant lowering of the coagulation time. If theelin therapy is an effective remedy, the proper dosage and interval of administration was not hit upon.—R. G. H.

Involutional melancholia; probable etiology and treatment. Werner, A. A., G. A. Johns, E. F. Hoctor, C. C. Ault, L. H. Kohler and M. W. Weis, J. A. M. A. **103:** 13. 1934. Abst., A. M. A.

The authors are treating 41 women with involutional melancholia from an endocrinologic point of view. Twenty-one of these women are being given 1 cc. of theelin intramuscularly daily. The others are being used as controls but will be given theelin later. The improvement in the theelin-treated patients has been greatly accelerated by the use of this hormone. The more rapidly beneficial results obtained in the theelin-treated patients seems to indicate that the administration of this hormone is rational and strikes at the fundamentally causative factor. The treatment recommended by leading writers is of a negative nature, such as guarding the patient against self-destruction, rest and quiet, sufficient sleep, maintenance of strength by nourishing food, and sedatives for nervousness and agitated states. The treatment as outlined is necessary but does not strike at any specific cause of the condition. The use of theelin in involutional melancholia, the authors believe, is a rational procedure, as the results seem to indicate, and that dosage larger than 1 cc. of theelin daily would produce beneficial effects more rapidly. Werner and Collier have shown that the clinical results obtained by theelin administration are dependent on the size of dosage and the duration of administration. The time element is an important factor in recovery. While apparently remarkable response is had within the short time of one or two months in an occasional case, this is not the rule. When a favorable response is manifested, the improvement is gradual and continuous, in contrast to the apparent slow improvement and regressions of patients previously treated palliatively and expectantly. Cases showing marked improvement are kept in institutions with difficulty, for the patients wish to return home when they feel more normal and the relatives are anxious to have them at home. When these patients become more rational, it is the consensus that most of them will do better under normal routine home life. However, their supervision and treatment with theelin should be continued at home under the care of the family physician for a period of one or two months after being symptom free to assist stabilization. If, after the patient has been off treatment, the condition should give evidence of recurrence, treatment should be reinstituted to prevent a relapse to the previous degree of mental incapacity. It might be well to give theelin to women who manifest severe menopausal symptoms and not wait for the more serious mental illness to overtake them.

Anterior pituitary-like principle in treatment of maldescent of the testicle. Cohn, S., J. A. M. A. **103:** 103. 1934. Abst., A. M. A.

The author treated four otherwise normal children for maldescent of the testicle with subcutaneous injections of anterior pituitary-like principle. Three cases evidenced complete improvement. The fourth case showed evidence of mechanical obstruction and will require operation. A fifth case presented one operated side, which furnished an admirable opportunity for a controlled observation, and showed evidences of pituitary hypofunction. Both the condition of the testicles and the general glandular hypofunction improved under the therapy. The sixth patient was operated on again after several years and injections of anterior pituitary-like principle were used as an adjunct to surgery. The result was entirely successful as to both the condition of the testes and the general condition of the patient.

Pituitary-thyroid syndrome associated with calcification of the pituitary body. Enfield, G. S., Pennsylvania M. J. **36:** 916. 1933.

This is a case report of a condition rarely encountered. The patient was a colored male of 17 whose chief complaint was drowsiness of approximately two years' duration. The family history and previous medical history were negative. Physical examination revealed sluggish reflexes, and an unduly palpable thyroid gland which gave a fibrous resistance, a pulse rate of 60 per minute and blood pressure of 90 systolic and 70 diastolic. The fundi were normal. A roentgenogram revealed the sella turcica to measure 14 mm. x 6.5 mm. The sella was traversed by a calcified network and the clinoids were

joined by a bony bridge. The basal metabolic rate was minus 17.8%. Other laboratory tests gave normal findings. The patient appeared to improve considerably under thyroid and pituitary opotherapy.—I. B.

A study of the pituitary factor increasing the ovarian weights of immature rats when injected in combination with pregnancy urine. Leonard, S. L., Am. J. Physiol. **108:** 331. 1934.

Littermate immature female rats were treated, one group with a pregnancy urine extract, one group with a gonadotropic extract of sheep hypophysis (the water soluble pyridine extract of Fevold, Hisaw and Leonard) and one group with a combination of the two (34 animals). The increases in weight of the ovaries of the immature rats produced by the combination of these gonadotropic substances was greater than the sum of the increases produced by each substance alone (from 110% to 319%). The hypophyseal extract was free of growth hormone when tested on dwarf mice and hypophysectomized rats. The synergistic action of these two hormones has been named "augmentation" by the Wisconsin group. Augmentation was obtained with pregnancy urine and alkaline extracts of beef, sheep, pig and human hypophyses (over 100 animals used). However, the degree of augmentation secured when the pituitaries of these several species were used was not the same. The human pituitary, which by itself is a very potent gonad stimulator, gave slight augmentation only with very small doses; the beef pituitary gave good augmentation only with large doses. Normal immature or hypophysectomized rats treated with a urinary follicle-stimulating hormone, and pregnancy urine presented a marked rapid increase in the size of the ovaries which was accompanied by luteinization of the follicles. It seems plausible that the synergistic reaction resulting from the combined injections is dependent on a differential action of two gonadotropic hormones which together produce a greatly enlarged ovary.—Author's Abst.

Responses of the reproductive system of hypophysectomized rats to injections of pregnancy urine extracts; the female. Leonard S. J. and P. E. Smith, Anat. Rec. **58:** 175. 1934.

Immature and mature hypophysectomized rats were injected with pregnancy-urine extracts. Treatments were begun both at operation and after a period had elapsed. Immediate treatments after hypophysectomy increased or maintained ovarian weights though regression occurred in prolonged injections. Postponed treatments invariably increased the weights of the ovaries. An hypertrophy of the interstitial and theca cells but not follicular growth was induced by the treatments. This hypertrophy did not persist in prolonged treatments except in certain newly-formed bodies which appear to be structurally identical with corpora lutea. These regressed but little, if at all, within the time limits of the experiments, thus behaving as do typically-formed corpora, present at the time of hypophysectomy. Corpora were not invariably formed in the immediate treatments in the younger prepuberal animals nor in the postponed injections. Oestrin was secreted in both the mature and immature hypophysectomized rats injected immediately after hypophysectomy. It was secreted also in some of the postponed treatments. The effects induced by P.U. injections in hypophysectomized rats are dissimilar to those produced by implants. In certain respects the effects of the injections are not the same in hypophysectomized as in normal rats. A.P. facilitates the action of P.U. as shown by results obtained in partial ablations and in concurrent injections of A.P. and P.U. in hypophysectomized and normal animals.—Authors' Abst.

Tumors of the hypophyseal region treated by radiotherapy (Tumeurs de la region hypophysaire traitees par la radiotherapie). Parfonry, J., Ann. d'ocul. **169:** 651. 1932. Abst., Arch. Neurol. & Psychiat. **30:** 1154.

Parfonry reports 3 cases in which tumors of the hypophyseal region were treated with radiotherapy (in 2 for 10 years, in the other for 4 years) in which improvement in the general condition, visual fields and visual acuity has been maintained throughout this period. He concludes from his observations that, although surgical treatment is important, it should be remembered that radiotherapy is of value when operation cannot be performed. Morax reported one case in which treatment was given with radiotherapy 20 times. Visual acuity was maintained for 7 years. Dupuy-Dutemps had previously reported several

cases which he had followed for more than 5 years. In his opinion, radiotherapy should always be given a trial before operation is advised. Magitot emphasized the fact that in one of the cases presented the visual field of one eye, which had been completely abolished, was totally restored. He also stated that in certain decompressions of the chiasm Cushing had seen the visual field restored on the operating table. Sourdille reported that he preferred operation. Bollack had previously reported 5 cases in which the condition was favorably influenced by radiotherapy, but he believes that operation offers the best chance for recovery.

Hypophyseal infantilism. Treatment with an anterior hypophyseal extract; preliminary study. Shelton, E. K., L. A. Cavanaugh and H. M. Evans, Am. J. Dis. Child. **47:** 719. 1934.

Shelton and his associates treated 6 children for a secretory deficiency of the anterior hypophysis. The diagnosis was presumptive in 5 cases and verified in one. Four patients were treated by the intragluteal injection of a growth-stimulating fraction of the bovine anterior hypophysis; one was given desiccated thyroid and desiccated whole pituitary by mouth; one was left untreated for 23 months and then given the growth-stimulating fraction of the bovine anterior hypophysis. The 2 latter patients served as controls. The growth increment of the 4 treated patients was considerably in excess of the expectancy, as computed from the average growth rate for the previous 4 years, and slightly in excess of the theoretical normal rate for their ages. One child changed in configuration and appearance. The growth increment for the 2 control cases was slightly in excess of the expectancy, but below the theoretical normal. The growth increment for the control case, in which the growth-stimulating fraction was given later, was in excess of the theoretical normal and several times the previous rate. The authors believe that the growth increment was greater in the first few months of treatment than subsequently, although the growth continued to equal or exceed the theoretical normal in all 4 treated cases. This may have been due either to a difference in the physiologic response or to the difference in the material. The authors do not consider that stimulation of growth has been proved, except in one case. Careful observation for at least a year is necessary to verify the results in the other children.—Authors' Abst.

Diabetes insipidus; treatment by intranasal insufflation of posterior lobe pituitary powder. Smith, F. M., J. A. M. A. **102:** 660. 1934. Abst., A. M. A.

The author observed 2 cases of diabetes insipidus of undertermined etiology under standard conditions to determine the relative effects of the usual treatment by subcutaneous injections of solution of pituitary and a modified treatment by intranasal insufflation of a powdered posterior lobe preparation. Intranasal insufflation of the powder in doses of from 40 to 50 mg. three times a day was shown to be as effective in maintaining a normal water balance with attendant alleviation of all symptoms as from 1.5 to 2 cc. of double strength solution of pituitary, administered subcutaneously. Advantages of the powder treatment include ease of application, absence of intestinal, cardiovascular or other side effects and, most of all, a reduction in cost to less than one-fifth that of solution of pituitary. A moderate dietary salt restriction is beneficial in the management of diabetes insipidus.

Insulin therapy in tuberculosis. Banyal, A. L. and G. H. Jurgens, Am. J. M. Sc. **188:** 76. 1934.

Observations on the effect of insulin in 43 non-diabetic patients with active, moderately and far-advanced pulmonary tuberculosis are presented. Insulin therapy was found to be of value in that it improved the appetite in 19 (79.1%) of the 24 moderately advanced cases and in 11 (57.8%) of the 19 far-advanced cases. It increased the weight in 13 (54.1%) of the moderately-advanced and in 9 (47.3%) of the far-advanced cases.—Authors' Summary.

Heredity as a factor in the etiology of diabetes mellitus. Cammidge, P. J., Lancet, **1:** 393. 1934.

Cammidge believes, as did Naunyn in 1906, that the underlying factor which unites the different clinical forms of diabetes mellitus is heredity, and that, given such an inherited tendency, any injury or disease throwing unusual

strain on the mechanism of carbohydrate metabolism may act as an exciting cause. He therefore analyzes a series of 1000 cases. Of these 39.6% showed an ancestral or family history of diabetes, an increase of 12% over the incidence found by the same author in a series of 800 cases published in 1928. The statistical results of his observations are given in two tables and one chart. He has determined the incidence of heredity and the proportions of mendelian recessives and dominants at each age period, comparing the figures with similar ones obtained on 500 non-diabetic patients. Apparently the recessive form predominates in diabetic patients developing the disease under 40 years of age, whereas in later life the disease seems to be more frequently transmitted as a dominant character. The author states that differentiation of the recessive from the dominant type is of some clinical importance since the dominant variety is almost invariably mild and is easily controlled by diet alone. The recessive form, on the other hand, is generally grave from the outset and frequently requires the use of insulin to keep it under control.

—H. C. Shepardson.

The effect of insulin upon body temperature (Experimentelle Untersuchungen uber den Einfluss von Insulin auf die Körpervörme). Düner, L., Arch. f. exper. Path. u. Pharmakol. **173:** 710. 1933. Abst., Chem. Absts. **28:** 1407.

Guinea pigs regularly have a fall in body temperature of as much as 2-3° within 1-2 hours after the injection of 2-3 units of insulin, not casually related to the hypoglycemia. The hypothermia is caused by the insulin itself, and not by the inert protein; crystalline insulin inactivated by H_2O_2 no longer causes hypothermia.

Relations of blood cholesterol to carbohydrate metabolism with especial consideration of diabetes mellitus (Uber Beziehungen des Blutcholesterins zum Kohlehydratstoffwechsel mit besonderer Berucksichtigung des Diabetes Mellitus). Grieshaber, H., Ztschr. f. klin. Med. **126:** 405. 1934.

A method for the determination of the blood cholesterol is described. It is carried out by the extraction with chloroform and by the photometric determination based on Liebermann's reaction. The results are reliable. The blood cholesterol was studied in diabetes mellitus in relation to the blood sugar, and also during glucose tolerance tests in normal subjects and diabetics. Experiments were carried out on rabbits with glucose, orally and intravenously, as well as with insulin and adrenalin. The results are as follows: (1) With every change in carbohydrate metabolism (administration of glucose orally and intravenously, insulin, adrenalin) there were characteristic changes in the blood cholesterol. (2) Insulin and adrenalin seem not to influence the blood cholesterol directly. (3) The degree of the disturbance in the carbohydrate metabolism in severe diabetes, and the effectiveness of the insulin therapy can be measured by the degree of the hypercholesteremia. Six charts and 12 tables are given.—A. Rudy.

Studies in diabetes mellitus; characteristics and trends of diabetes mortality throughout the world. Joslin, E. P., L. I. Dublin and H. M. Marks, Am. J. M. Sc. **186:** 753. 1933.

Analysis of available mortality data on diabetes in this country and abroad during the present century brings out these main features. Diabetes as a cause of death has been rapidly growing in importance during the present century and is now one of the leading causes of death in this country. The mortality from diabetes in this country is higher than in other parts of the world. Diabetes death rates are increasing all over the world. Most of the deaths from diabetes occur after middle life. Among children and young adults, the mortality from the disease is low. It begins to loom large about age 40 and the rise in the death rates by age thereafter is rapid. Up to age 35 present death rates of males and females are little different, but after that age the rates of women are the higher. This excess of the female death rate increases with advancing age up to 65, when it is about twice that of males. The high diabetes mortality in this country, as compared to other countries is not limited to one sex, but is found among both men and women. It is also found to exist at almost every age past 35. The diabetes mortality among negroes in this country has been increasing at a more rapid rate than among

whites, and is now not much lower than that of whites. The diabetes mortality of negroes is distinguished by relatively higher death rates in early adult and middle life. At all ages combined, the trends in diabetes death rates over the past two decades among males in this country and England have been slightly downward, although there has been a tendency in recent years for these rates to increase. In most other countries, there has been a moderate increase in the long-term trends of mortality from the disease. Among females, however, there has been a substantial rise in diabetes mortality, both in this country and abroad. Analysis of death rates by age, however, shows that in childhood and in early adult life there has been distinct improvement both among males and females. In the case of men, this improvement extends right through middle life and into the early part of old age. Among women, improvement ceases by age 50 and there are substantial increases in the death rates during late middle life and throughout old age. This analysis of the diabetes situation in this country and abroad clearly indicates that the problem of its increasing mortality is circumscribed. It is one of controlling the disease in older people, chiefly in women. The increase in diabetes is thrown into sharp relief because it is just these groups which are increasing most rapidly in our population. On the other hand, it is certain that there has been a substantial growth in the incidence of diabetes throughout the world. We are forced to this conclusion because the mortality from diabetes has been increasing in the face of unmistakable improvement in the treatment of the disease.

—Authors' Summary.

On the inheritance of diabetes mellitus; further analysis of family histories. Pincus, G. and Priscilla White, Am. J. M. Sc. **188**: 159. 1934.

Among the parents and siblings of a group of diabetic patients the reported incidence of diabetics is 300 in a total of 4434 individuals (6.7%), whereas only 16 diabetics were reported in a total of 1296 parents and siblings of a group of non-diabetic patients (1.23%). The difference between the percentages observed is statistically significant. When the assumption is made that diabetes develops in individuals homozygous for a recessive gene (i.e., in mm individuals), it can be demonstrated that the ratios of diabetic to non-diabetic individuals among the siblings of the diabetic patients conform with the consequent expectations, provided we assume that potential diabetics before they develop the disease are subject to the ordinary chances of death. Employing differing incidence data causes little variation in our mendelian expectations. This is taken to indicate that as long as diabetes occurs in the fairly regular manner indicated by ordinary incidence tables, and as long as the chances of death indicated by the ordinary life tables apply, mendelian expectations will be satisfied. It is, therefore, highly probable that the development of diabetes mellitus depends primarily upon the transmission of a single recessive gene. But the ordinary age-incidence relations indicate the operation of one or more secondary factors. Our data give no accurate clue to the nature of these secondary factors.—Authors' Summary.

Pathogenesis of diabetes mellitus (Untersuchungen zur Pathogenese des Diabetes mellitus). Pollak, L., Wien. klin. Wchnschr. **45**: 257. 1932.

A study of galactose assimilation in normal and diabetic persons was made by a series of simultaneous determinations of galactose and glucose in the blood, following the ingestion of 40 grams of galactose. The galactose tolerance of the diabetic is even better than that of the normal, while the glucose tolerance is impaired. This contradictory factor is called the galactose paradox in diabetes. Hyperglycemia of any origin, alimentary or following adrenaline hypersecretion, hastens the assimilation of galactose. The diabetic hyperglycemia is the reason why the galactose tolerance in diabetes is normal, or even increased in spite of the lack of insulin. This explanation is valid even in case the diabetic hyperglycemia should in the long run be due to a relative hypersecretion of adrenaline, as believed by some. In all diabetics following the administration of galactose the blood sugar level rises and often remains high for some time. This points to an abnormally strong pouring out of glucose from the liver. With the aid of the ergotamin test the impulses which stimulate the secretion of glucose from the liver can be divided in two groups, the same as in non-diabetics, ergotamin sensitive and ergotamin refractory. Both kinds of impulses are found in all forms of diabetes. The impulses for the secretion of glucose are of the same kind and strength in the diabetics as in

the non-diabetics. In the diabetics they produce a greater rise of the blood sugar level, because the inhibiting effect of the insulin, which lowers the sensitiveness of the liver, is missing in all forms of diabetes. The fact that in diabetics ingested galactose does not produce a drop in the blood sugar as in normal individuals, would suggest a lack of insulin. The stimulating effect of galactose on the various hormones, as seen in the experiments, is used as another proof of the pharmacologic action of food. Nine charts illustrate the effect of ingested galactose on normal and diabetic individuals and the influence of ergotamin and atropin on the galactose test.—A. Rudy.

Association of diabetes and tuberculosis. Root, H. F., New England J. Med. **210:** 1. 1934.

The paper is divided into 4 parts covering epidemiology, pathology, clinical features, and treatment with a summary at the close of each section. The various phases are considered fully. Two points make a knowledge of the relationship of the two diseases of importance. Tuberculosis occurs much more frequently in diabetics than in the rest of the community. Since the tuberculosis usually develops after the diabetes, it is very apt to be overlooked in its early phases.—J. C. D.

Hyperinsulinemia secondary to an adenoma of the pancreas. Ross, L. I. and J. M. Tomasch, Arch. Surg. **28:** 223. 1934.

Ross and Tomasch report the clinical and laboratory data on a case of spontaneous hyperinsulinemia with resultant hypoglycemia. The blood sugar level during the attack dropped to 23 mg. per 100 cc. Following surgical removal of a small adenoma of the island tissue of the pancreas the patient's carbohydrate metabolism was restored to normal and he was entirely relieved of his hypoglycemic seizures. Post-operative fasting blood sugar values ranged from 99 to 151 mg. per 100 cc. The pre-operative glucose tolerance test showed, as in most of the reported cases, a diabetic type of response but the final post-operative test showed a normal curve. This case, which at the time observed was the fifth thus far reported, differed from the previously recorded cases in several respects. The patient had had no premonitory symptoms or preceding mild attacks. Because of the abrupt onset of symptoms with associated evidence of external injury, his confused state was at first attributed to cerebral oedema or concussion. The authors likewise noted the semi-Babinski or extensor response of Hart and Bond during each of the hypoglycemic seizures. This response has been cited by Hart and Bond as a diagnostic aid in differentiating between insulin shock and diabetic coma. Exploratory laparotomy with excision of the adenoma, if demonstrable, is the treatment of choice in these cases. Follow-up examination on March 14, 1934, two years and four months after operation, showed the patient to be entirely well without any symptoms or complaints referable to hypoglycemia. Fasting blood sugars ranged from 83 to 97.5 mg. per 100 cc. of blood. The glucose tolerance test showed a normal curve.—L. I. Ross.

Inheritance of diabetes. White, Priscilla, E. P. Joslin and G. Pincus, J. A. M. A. **103:** 105. 1934. Abst., A. M. A.

The authors found that each member of similar twins developed diabetes more than four times as frequently as each member of dissimilar twins; namely, 70% of the former and 16% of the latter. The incidence of diabetes in the relatives of a diabetic population is significantly greater than in a non-diabetic group. Mendelian ratios were found in a consecutive series of diabetic cases and in a selected mendelian population tested by histories and blood sugars. Many blood relatives of diabetic patients have symptomless hyperglycemia, the significance of which is unknown. These instances occurred when one would expect diabetes to develop according to mendelian inheritance. The authors believe that the potentiality for developing diabetes is transmitted as a simple mendelian recessive trait and that the secondary factors which permit the expression of the gene can best be studied among predestined diabetic patients; namely, homologous twins of diabetic patients and the offspring of two diabetic patients.

The changes in the concentration of inorganic calcium and phosphorus during convulsions of experimental origin, in cats, before and after thyroparathyroidectomy, with and without bromide therapy. Coombs, Helen C., D. S. Searle and F. H. Pike, Am. J. Psychiat. **13:** 761. 1934.

The minimal convulsant and total dosage of a drug necessary to elicit convulsions is much less in cats after thyroparathyroidectomy than in control animals. The number of electrical excitations of the cortical motor area possible in cats after thyroparathyroidectomy is much smaller than in controls. The number of occlusions of the head arteries which can be done in animals after thyroparathyroidectomy is much smaller than in controls. The administration of sodium bromide for two weeks before thyroparathyroidectomy prevents the post-operative appearance of tetany and, if continued, prolongs life for at least three or four weeks without other remedial measures. In cats after thyroparathyroidectomy, the calcium phosphorus ratio is almost 50% lower than in controls; the calcium phosphorus ratio in cats under bromides before operation is, however, less changed, and may even approach the ratio of the controls. While there is always a rise in calcium and phosphorus of the blood serum following experimentally induced convulsions, both in controls and after simple thyroparathyroidectomy, previous administration of bromides causes a fall of calcium and phosphorus following convulsions, in both sets of conditions.—Authors' Summary.

Calcium and phosphorus metabolism in diseases of the thyroparathyroid apparatus. Hansman, F. S. and F. H. Wilson, M. J. Australia, **1:** 37. 1933. Abst., J. Nerv. & Ment. Dis. **79:** 450.

The thesis of Aub and his co-workers and of Hunter that thyroxin has a direct catabolic effect on the calcium deposits in the bones cannot be accepted on the evidence they present. Seven patients suffering from hyperthyroidism have been carefully studied. An analysis of their experimental data provides definite evidence in favor of an associated hyperparathyroidism being the direct cause of the excessive mobilization and excretion of calcium and phosphorus. Two patients suffering from hyperthyroidism with an associated hypoparathyroidism were studied. Both patients were in calcium and phosphorus equilibrium. Though hyperthyroidism is frequently accompanied by a negative calcium and phosphorus balance, it is not invariably so. It is possible for calcium and phosphorus equilibrium or a positive calcium and phosphorus balance to be present. Hyperthyroidism per se has no specific effect on calcium and phosphorus metabolism. We were unable to confirm the findings of Aub and his co-workers and of Hunter that the excretion of the mobilized calcium is predominantly fecal.

Growth, reproduction and lactation in the absence of the parathyroid glands. Kozelka, F. L., E. B. Hart and G. Bohrstedt, J. Biol. Chem. **100:** 715. 1933.

The authors studied the effect of thyroparathyroidectomy on puppies. Diet consisted of a ration adequate in protein, carbohydrate and fat, but with high and low calcium content in separate animal groups. Vitamin D was used to support the normal values of calcium in the blood. Thyroid medication was given (0.120 gm. per day). Tetany was used as the criterion of totality of the parathyroid ablations. It was found that dogs whose calcium level was adequately controlled by proper dosage of Vitamin D (irradiated ergosterol 1000 D), not only lived on without the development of tetany, but grew normally, had normal oestrus, successful gestation and lactation. Gestation and lactation markedly increased the requirement of Vitamin D, as judged by the dosages needed to support the blood calcium levels at normal values. Such dosages were found to be distinctly toxic when administered to normal, non-pregnant dogs and regularly produced hypercalcemia, even in the absence of the parathyroid glands. They caused calcium deposits in tissues such as lung, kidney, and so forth (tissue analyses given). Clinical symptoms of overdosage with Vitamin D paralleled the development of hypercalcemia. The authors conclude from their analyses of the calcium and phosphorus of ashed tissues that Vitamin D has the power of direct liberation of calcium from the bones in a manner quite similar to that of parathormone. The authors infer that the parathyroid glands, while they serve to regulate the calcium and phosphorus levels of the blood of intact animals, have no specificity in that function; and that their secretion can be supplanted by Vitamin D with no interference with

growth, oestrus, gestation or lactation, as well as in the experimental production of hypercalcemia and the prevention of tetany in the parathyroidectomized animal.—S. Hertz.

Investigations of the action of parathyroid extract on the skeleton (Recherches sur l'action de l'extrait parathyroïdien sur le squelette). Leriche, R., A. Jung and S. Cemil, Presse méd. **41**: 2059. 1933. Abst., Surg., Gynec. & Obst. **59**: 76.

In experiments on animals the authors found it possible to produce a clinicopathological syndrome resembling that of von Recklinghausen's disease of bone by injecting parathyroid extracts. The experimental animals were rats from three to four weeks old, weighing from 45 to 50 gm. Of the various parathyroid extracts used, Collip's parathormone was found to be the most effective. After eight daily injections of 20 units of this preparation, a fibrosis of the marrow of the femur in the diaphyseal and metaphyseal regions was observed. Haematopoietic marrow disappeared. In the cancellous bone and cortex in the metaphysis and diaphysis lacunar resorption occurred and the haversian canals became wider than normal. Roentgenograms after the twelfth day demonstrated a marked reduction in the density of the medullary portions and a thinning of the cortex. Other preparations of the parathyroid glands did not produce such marked changes until after a much longer period and after the administration of much larger doses. When paratyrone was used, daily injections of from 20 to 40 units for from one week to two months were required to produce lacunar absorption of bone and no fibrosis of the marrow occurred. All of the parathyroid extracts produced hypercalcaemia, but there were marked differences in the cellular changes in the bones brought about by the different preparations. The authors therefore conclude that the type of extract used is of prime importance in both experimental and clinical work. The parathyroid glands of the injected animals presented no gross abnormalities, but on microscopic examination showed disappearance of blood capillaries roughly in proportion to the amount of extract injected. After many injections a definite sclerosis of the gland was produced and the normal granular appearance of the cytoplasm of the parathyroid cells were modified. The nuclei were changed, appearing elongated or triangular instead of round or oval. These findings were interpreted by the authors as suggesting degeneration of the gland due to an oversupply of the parathyroid hormone.

A case of hyperparathyroidism with certain unusual features. Mimpriss, T. W. and R. W. Butler, Brit. J. Surg. **21**: 500. 1934. Abst., Surg., Gynec. & Obst. **58**: 557.

The authors report in considerable detail a case of hyperparathyroidism with an associated parathyroid tumor approximately 12 mm. in length embedded in the anterior surface of the thymus. The patient was a boy 17 years of age. The symptoms were pain in the knees causing difficulty in walking, pain in the long bones brought on by pressure, general retardation of growth and defective mentality. When the patient was first seen there was no evidence of kidney insufficiency, but this became marked later in the disease. The calcium content of the blood varied between 14 and 18 mg., and the phosphorus content between 3 and 4 mg. per 100. X-ray examination of the bones revealed several areas of decreased density in the metaphyses similar to those of osteitis fibrosa and a well-defined transverse band of increased density in the metaphyses immediately adjacent to the epiphyseal line. The authors suggest that this band of increased density, which has not been previously described as a finding in hyperparathyroidism, may be associated with the presence of active epiphyseal growth. Most of the cases of hyperparathyroidism on record occurred between the ages of 30 and 55 years, when the epiphyseal lines were closed. Excision of the parathyroid tumor in the authors' case was followed by complete recovery. Six months later, x-ray examination of the bones revealed no abnormalities, and the chemical character of the blood was normal.

An experimental study of the histology and cytology of the parathyroid glands in the albino rat. Rosof, J. A., J. Exper. Zoöl. **68**: 121. 1934.

Parathyroid glands extirpated from 275 albino rats were studied by means of histological and cytological methods. Changes in the Golgi apparatus, mitochondria, cytoplasm and cell counts were correlated with parathyroid activity

and with factors which are said to influence the susceptibility of the white rat to fatal parathyreoprival tetany. It was found that the condition of the mitochondria and Golgi apparatus varied with and was related to the secretory activity of the cells. Secretory polarity was indicated by the Golgi apparatus. The secretory cycle was divided into three stages, the secretory stage being indicated by cells, which darkened with osmic acid. These cells usually form less than 25% of the total number of parathyroid cells. The average parathyroid cell counts of 60-day rats, fatal tetania parathyreoprival cases, and underweight animals are less than the average cell counts of the 90-day rats, non-fatal tetania parathyreoprival cases and overweight or normal weight animals, respectively. No significant differences were found in the average parathyroid cell counts of overweight and normal weight animals; hyperthyroid and normal or hypothyroid animals; summer and winter parathyroidectomy operative material. Parathyroid gland cells that remain after incomplete parathyroidectomy do not undergo a compensatory hypertrophy. Cell size is not correlated with gland size in the 60-day rats but in the 90-day rats gland size is directly proportional to cell size.—Author's Abst.

Calcium and phosphorus studies; effect of calcium and food intake of parathyroidectomized rats. Shelling, D. H. and Dorothy H. Asher, J. Biol. Chem. **96:** 195. 1932.

Parathyroidectomized rats were kept on diets in which the content of phosphorus and calcium were varied. Diets high in calcium or low in phosphorus ameliorated tetany; high dietary calcium gave high serum calcium. Diets low in calcium or high in phosphorus were conducive to tetany, anorexia, and cachexia. The compositions of the various diets and the resulting concentrations of calcium and phosphorus in the serum are tabulated. The form in which phosphorus occurs in the diet proved to be important: sodium metaphosphate or hypophosphite gave no detectable effects, while pyrophosphate and orthophosphate had the same effects as the usual forms of dietary phosphorus.
—F. T. Jung.

Studies on the physiology of the thyroid gland; the iodine-containing substance of the thyroid (Studien zur Physiologie der Schilddrüse). Blum, F., G. A. Lehmann and W. Leistner, Endokrinologie, **13:** 250. 1933.

Thyroglobulin was extracted from the thyroid glands of hogs, sheep, and humans. Such material could be separated into two fractions: (A) by adding saturated ammonium sulfate solution up to 56 volumes per cent; and (B) by adding saturated ammonium sulfate above 65% (by volume). The two fractions were further purified by dialysis, followed by reprecipitation with saturated $(NH_4)_2SO_4$. Contaminating nucleoproteid also was found in both fractions. Fraction (A) dissolved slowly, forming a colloidal solution; but fraction (B) dissolved readily to form an apparently simple (crystalloid-like) solution. Generally, (A) was greater in mass than (B). For example, 100 sheep glands yielded (A) 2.63 grams of material, containing 1% iodine; and (B) 1.25 grams, containing 0.6% iodine. Corresponding percentages from human glands were 0.47% and 0.33%, respectively. There was considerable seasonal and individual variation in these values. The highest figures obtained without preliminary iodine-feeding were (A) 1.2% and (B) 0.76%.—W. T. Salter.

Hypothyroidism and cretinism in childhood; mental development. Bronstein, I. P. and A. W. Brown, Am. J. Orthopsychiat. **4:** 413. 1934.

A preliminary report is given of a study of the intellectual level in a group of 21 children (8 boys and 13 girls) from one to 16 years of age who were receiving active treatment with thyroid extract for hypothyroidism. Various psychometric tests were used, chiefly the Stanford revision of the Binet-Simon test. The tests were repeated at irregular intervals over a period varying from one month to five years. The intelligence quotient (I.Q.) as determined by the Stanford revision of the Binet-Simon test in 17 of the subjects ranged from 43 to 107, with an average of 65. At a second examination on 14 of these 17 subjects the I.Q. ranged from 38 to 102, with an average of 60. The test was given a third time to eight subjects and a fourth time to six subjects. The I.Q. remained fairly constant from one examination to another, the average difference between the first and last examination being 5.76. In some of the subjects studied the hypothyroidism had been recognized early and adequate active treatment was carried out. In others, the hypothyroidism had not been

recognized early or active treatment had not been begun and carried on continuously. It is suggested that prompt recognition and persistent treatment gives the best results in both physical and mental development; that children who have normal birth and developmental histories and develop thyroid deficiency later in life following infectious diseases attain a good intellectual level if properly treated; that there are individual variations in the intellectual level reached by children with hypothyroidism under active treatment.
—H. T. Carmichael.

The carbohydrate metabolism in hyperthyroidism and in simple goiter; influence of operation. Chiba, T., Tohoku J. Exper. Med. **23:** 474. 1934.

The author studied the glucose tolerance test by giving 50 gm. glucose on fasting stomach and 50 gm. 1.5 hours later in a series of 14 cases. He concludes from his microchemical studies of the blood sugar that in hyperthyroidism the average fasting blood sugar levels are normal, 80 to 111 mg. per cent. The ability to utilize sugar in these cases is, however, disturbed according to him, and presents a hyperglycemia of a more pronounced and longer type than that found in the normals. This ran according to the severity of the hyperthyroidism. The simple goiter series showed no deviations from the normals. Operative intervention had a beneficial effect on the carbohydrate metabolism and the improvement was parallel with the improvement of the clinical symptoms.—H. J. J.

Place of iodine in the treatment of goiter. Clute, H. M. and L. S. Pilcher, New England J. of Med. **210:** 117. 1934.

Three hundred and fifteen patients suspected of having thyroid disorders were seen during 1931 and 1932. Of these, 216 showed actual thyroid changes. The authors conclude that iodine in small doses is of the greatest importance in the prevention of endemic goiter in adolescent children living in goiter belts. Iodine is of the greatest value in the preparation of patients with hyperthyroidism whether it be primary or secondary for the surgical removal of the thyroid gland. Iodine may do considerable harm in misleading the patient and postponing, in certain cases, the necessary surgical measures in potentially malignant non-toxic goiters. The administration of iodine for long periods of time in hyperthyroidism does great harm in increasing the risk of surgery, the number of operations necessary for the relief of the patient, and in the prolongation of the patient's disability.—J. C. D.

Acute thyroiditis with report of ten cases. Cochrane, R. C. and S. J. G. Nowak, New England J. Med. **210:** 935. 1934.

The local signs and symptoms overshadowed the endocrine aspects of the three patients who died. Only one showed systemic disturbances due to the thyroid. The treatment was palliative in six cases and surgical in four.
—J. C. D.

Lipodystrophy in adults. Cohen, S. J. and B. M. Eis, Arch. Neurol. & Psychiat. **32:** 184. 1934.

Two cases of lipodystrophy with hyperthyroidism, mental symptoms and a disturbed cholesterol metabolism, occurring at the menopause, are reported. The symptoms and signs of lipodystrophy in adults are different from those in children. Thyroidectomy does not relieve the signs and symptoms of hyperthyroidism when it is associated with lipodystrophy. Lipodystrophy is probably dependent on endocrinopathy.—Authors' Summary.

A prepotent factor in the determination of skull shape. Dye, J. A. and F. S. Kinder, Am. J. Anat. **54:** 333. 1934.

Total thyroidectomy was performed on 10 puppies 3 to 4 weeks of age, 8 littermates were kept as controls. After a survival period of 3 to 8 months the animals were sacrificed and the skulls prepared for careful measurement and study. The skulls of the thyroidectomized animals, as compared with those of their littermate controls, showed marked retardation of ossification and ankylosis of the bones developing from cartilate—the ethmoid and basal bones of the skull—while those developing from membrane were little or not at all affected. Restricted bone growth in these areas exerted a marked influence on cranial development. The bases of the skulls of thyroidectomized animals

were relatively short while, due to subnormal development of the ethmoid in each, the root of the nose was depressed. As a result of these abnormalities cranial growth was passively altered. To accommodate for the developing brain, expansion of the cranium took place laterally and occipitally which resulted in the development of a marked brachycephaly.—Authors' Abst.

The medical care of patients following total thyroidectomy. Eppinger, E. C. and S. A. Levine, J. A. M. A. **102:** 2076. 1934. Abst., A. M. A.

The authors give the three complications with which the physician is concerned in the postoperative care of patients subjected to complete thyroidectomy. 1. Hoarseness and aphonia as a result of injury to the recurrent laryngeal nerves is rare and requires no specific treatment. 2. Postoperative tetany is quite uncommon, is never severe and is relieved by the administration of viosterol and calcium. 3. Mild symptoms of myxedema occur almost invariably, are not necessarily paralleled by a low basal metabolic rate, and can be controlled easily by thyroid medication. It is generally desirable to keep the metabolic rate at a level of about minus 20. It was found that smaller doses of thyroid extract were necessary to produce the same effect in surgical myxedema than in the spontaneous type. These patients should be given the same sort of medical advice as is given to cardiac patients in general, except so far as improvement permits an increased range of activity.

Experimental exophthalmos and hyperthyroidism in guinea pigs; clinical course and pathology. Friedgood, H. B., Bull. Johns Hopkins Hosp. **54:** 48. 1934.

Simultaneous studies of the basal metabolic rate and pathology of the thyroid gland were carried out on 30 guinea pigs injected with anterior pituitary extracts over a period extending from 48 hours to 190 days. Six guinea pigs were used for controls. These injections produced a syndrome which is remarkably similar to that of exophthalmic goiter in man, but there is no evidence at present that the pathogenesis of these two conditions is identical. This experimental state is characterized principally by exophthalmos, hypertrophy and hyperplasia of the thyroid gland, and a coincidental increase in the rate of oxygen consumption. The latter reaches a maximum elevation (+26% to +60% above the normal) between the 7th and the 14 day of the experimental period, and maintains this new level of metabolism for a few days after which a spontaneous remission, extending over a period of one to three weeks, results in a decrease of the basal metabolic rate to its original value or to a point somewhat below normal. This spontaneous remission may be due to a relative functional insufficiency of the thyroid gland, although the development of a protective "anti-pituitary" or "anti-thyroid" substance must be considered a possibility. Parenchymal hypertrophy and hyperplasia of the thyroid gland appear simultaneously with systemic evidence of its hyperactivity, but these same pathological changes may still be present for a time when the basal metabolic rate is spontaneously decreasing. The exophthalmos is produced independently of the calorigenic hormone of the thyroid gland, and is even more prominent in the absence of hyperthyroidism. Chronic exophthalmos may result from prolonged injections.—Author's Abst.

Psychic trauma in the pathogenesis of Basedow's disease (Das psychische Trauma in der Pathogenese der Basedowschen Krankheit). Geyer, H., Ztschr. f. klin. Med. **124:** 168. 1933.

This is a brief review of the subject with the inclusion of a number of illustrative case histories. Evidence is presented establishing a causal relationship between psychic trauma and Basedow's disease. Whether psychic trauma plays a direct or merely a contributory rôle in the genesis of the syndrome is not clear. Analysis of every case of Basedow's disease apparently induced by psychic trauma, reaching the Kiel Clinic between the years 1925 and 1931, permits of the division of psychic trauma into two types: acute through shock and the like, and chronic, originating in intense emotionalism.—I. B.

Differential diagnosis of hyperparathyroidism. Gutman, A. B., P. C. Swenson and W. B. Parsons, J. A. M. A. **103:** 87. 1934. Abst., A. M. A.

The authors present four cases of hyperparathyroidism. Data on 115 published proved cases of hyperparathyroidism indicate that the disease occurs more frequently in females (86 females, 29 males) and, while widely distrib-

uted as to age incidence, occurs most frequently in middle life. The course of the disease is usually measured in years, rarely in months. The authors state that hyperparathyroidism should be considered in the presence of obvious skeletal manifestations such as spontaneous fractures, bone swellings particularly of the jaws, gross deformities, and unexplained persistent pain in the bones. Obscure cases with presenting symptoms of marked polydipsia and polyuria, recurring renal calculi or unexplained asthenia with wasting of the muscles occasionally prove to be parathyroid adenomas. In most such cases the diagnosis of hyperparathyroidism may be confirmed or excluded by consideration of the history and clinical manifestations, roentgen study of the bones, increased serum calcium and phosphatase activity, low serum inorganic phosphorus and mineral balance studies. The results of clinical or metabolic or roentgenologic investigation alone may be indecisive or misleading. This is particularly true in the presence of marked renal insufficiency, or when the disease is in its early stages, is only mildly progressive or is in remission.

The preparation and properties of thyroglobulin. Heidelberger, M. and W. W. Palmer, J. Biol. Chem. **101:** 433. 1933.

Aqueous extracts of hog thyroid glands contain a fraction precipitable in the cold at pH 4.8 to 5.0, which has the properties of a nucleoprotein. When saline extracts, prepared according to Oswald's classical procedure, were precipitated with acetic acid or with $(NH_4)_2SO_4$ at half-saturation, this impurity was found in the product obtained by either method. It was difficult to remove this contaminant because of the rapid denaturation which thyroglobulin undergoes at a pH below 4.8 even in the cold. On denaturation, the range of minimum solubility of thyroid protein shifted from pH values of 3.8 to 4.3 upward toward the range of acidity in which the impurity separated. This rapid alteration tended to make mixtures of thyroglobulin and the contaminant inseparable.—W. T. Salter.

The effect of varying levels of iodine intake on the thyreoglobulin content of the thyroid gland. Jones, Mildred Elinor, Am. J. Physiol. **107:** 513. 1934.

Iodine in the form of KI fed to dogs produced an increase of 146.8% to 249.22% in thyroid iodine, 94% to 99.9% of which was in a form precipitable by 95% alcohol, and therefore, probably thyreoglobulin. Dosages of 0.02 gram per kilo. daily for 4 weeks were as effective in producing iodine storage as those of 0.1 gram daily for 6 weeks. The biological activity per unit of iodine in the thyroid gland as found by the Feather Germ test is of the same order of magnitude after as before iodine administration.—Author's Summary.

Thyroid disease. Lahey, F. H., New England J. Med. **210:** 1016. 1934.

In children hyperthyroidism can be treated surgically with excellent results, if the operation is performed in two stages and care used not to remove too much of the gland. Exophthalmos represents a serious risk to the eyes. Post-operative exophthalmos associated with myxedema can be treated by orbital decompression. One type of hyperthyroidism lacks most of the usual symptoms and may be described as "apathetic hyperthyroidism." It is best dealt with by a two-stage operation. Another type is the intra-thoracic goiter, which can be detected by x-ray. The general practitioner should be familiar with the proper method of dealing with thyroid crises. Intravenous fluids and iodine are used. Operations should follow within two or three weeks of recovery. In 12,246 thyroid operations, the mortality was 0.73%. Since 1926, however, the rate for primary hyperthyroidism has been 0.48% in 4,543 operations and for toxic adenoma 1.5% in 1032 operations.—J. C. D.

Further observations on the heart in myxedema. Lerman, J., R. J. Clark and J. H. Means, Ann. Int. Med. **8:** 82. 1934.

The changes in the size of the heart and in the blood pressure of 18 patients with myxedema under thyroid medication are presented to supplement the data of 30 patients previously reported. It appears to be characteristic of the heart in myxedema to be enlarged and to undergo appreciable shrinkage as the disease is ameliorated by the administration of thyroid substance. All of the electrocardiograms of these patients taken before treatment showed abnormalities. None of the patients showed congestive failure.

—Authors' Summary.

The blood picture in hyperthyroidism and in hypothyroidism. McCullagh, E. P. and J. H. Dunlap, J. Lab. & Clin. Med. **17**: 1060. 1932.

An analysis of 1200 consecutive routine blood counts and 250 differential counts in hyperthyroidism was made. The red and white blood cell counts were normal. The haemoglobin was slightly reduced. There was an absolute and relative lymphocytosis and slight absolute and relative decrease in the number of polymorphonuclear neutrophiles. The relative lymphocytosis bore a close relationship to the total white blood cell count and to the absolute number of neutrophiles. It bore little or no relationship to the severity of the disease as gauged by the basal metabolism. Comparison of 28 patients with hyperthyroidism before and after thyroidectomy revealed a decrease in both the relative and absolute number of lymphocytes; there was a definite rise in the relative and absolute number of neutrophiles. Twenty patients with hyperthyroidism were examined more carefully; 12 patients were studied before and after thyroidectomy; similarly, 20 cases of post-operative myxedema were studied. In this latter group the average red count, hemoglobin, color index, volume index and saturation index were all slightly below normal. The white blood cell count and differential count were about the same as in hyperthyroidism. In 10 cases of hypothyroidism a filament count was about 19%. In 10 cases of hyperthyroidism the filament count was about 12% both before and after operation. The findings obtained by careful examination of the blood were about the same as those obtained by routine blood studies, except that in the former the leucocyte count was slightly lower, the relative lymphocyte count was slightly higher and the relative polymorphonuclear count was slightly lower.—J. Lerman.

End results in exophthalmic goiter; patients treated in pre-iodine days. Pittman, H. S., New England J. Med. **210**: 912. 1934.

Reports of 94 patients treated between 1913 and 1923 were obtained in 1933. Of these, 69 are well, 8 have mild hyperthyroidism and 7 myxedema. The results of treatment by surgery or x-ray alone were about equally good. A combination of the two was the most effective with 86% cures. It took six months to a year usually before symptoms were relieved. This is in marked contrast to the rapid relief following subtotal thyroidectomy in iodinized patients.—J. C. D.

Experimental contribution to the Reid Hunt (acetonitrile) reaction with especial consideration of the sera from pregnant, eclamptic and carcinomatous women and extracts from the urine of pregnancy ("Experimenteller Beitrag zur Reid Hunt-Reaktion mit besonderer Berücksichtigung der Sera von Schwangeren, Eklamptischen und Karsinomkranken und Extrakte aus Schwangerenharn"). Sommer, S., Zentralbl. f. Gynäk. **58**: 385. 1934.

Active extracts of the anterior lobe of the pituitary and serum from pregnant women produce histological evidences of increased activity in the thyroids of experimental animals while pregnancy in the guinea pig produces the same end result in that animal. Using the acetonitrile reaction of Reid Hunt, as a measure of thyroid activity, several investigators have reported wholly contradictory results. Using white mice as the experimental animals, the author has studied the thyroid-stimulating effect of sera from late normal and eclamptic pregnant women, those with carcinoma of the pelvic contents and also several active preparations from the urine of pregnancy. The animal colony was carefully standardized by a preliminary control rest period and the minimum lethal dose of acetonitrile (amount not given) determined which would usually cause death in 24 hours. Experimental series of standardized mice (5 animals) were given three doses of 0.5 cc. of serum or equivalent amounts of the other materials within a test period of 72 hours and then 24 hours later graded doses of acetonitrile (1, 4/3, 5/3, 2 times the lethal dose) administered. The sera from the carcinomatous patients and normal controls gave no protection, the several urine extracts slight benefit (Prolan and Präpitan at 3/3 level, Pregnyl and Prähormon at 4/3), serum from eclamptics in the ninth lunar month protected against the poison to the 5/3 dose and normal sera in the last two months of pregnancy doubled the resistance of the mice. Serum from intact injected rabbits will protect white mice, while that from thyroidectomized rabbits is valueless. Direct injection of the thyreotropic hormone into thyroidectomized guinea pigs is without effect against the acetonitrile

test. The author concludes that in the serum of pregnancy there is not only the thyroid hormone but also the thyreotropic product of the anterior pituitary and this latter is the active agent in producing the relative immunity of the injected experimental animal to the toxic action of acetonitrile.—A. W. R.

Transplantation of living grafts of thyroid and parathyroid. Stone, H. B., J. C. Owings and G. O. Grey, Lancet, **1**: 625. 1934. Abst., J. A. M. A. **102**: 2067.

Stone and his associates believe that in the transplantation of living grafts of thyroid and parathyroid tissue a proper site should be selected and the form and size of the grafted material, the adaptation of the graft to the chemistry of its host and the existence of a state of deficiency causing a physiologic need of the tissue to be grafted should be considered. They have secured cross grafts of thyroid and parathyroid tissue which, when removed, seemed to be entirely healthy and growing vigorously at varying periods from 21 to 80 days after the implantation. There have been a great many failures, but the proportion of success has been sufficient to show that cross grafting of these endocrine glands is possible when all the conditions of the experiment are favorable. The method has been applied to two cases of human parathyroid tetany and four cases of thyroid deficiency. One of the tetany patients has lived one year since the first graft and the other seven months. They are entirely well clinically, and their blood calcium has returned to normal from a low level of 4.6 and 6.2 mg. per hundred cubic centimeters, respectively. The thyroid cases are too recent to make any report on. The details of the technic of tissue culture for these grafts of endocrine glands follow in general, the principles well established for the growth of mammalian tissue. A small fragment is placed, under sterile conditions, on a coagulated medium and kept in a thermostat until it begins to liquefy the surrounding medium by its growth and chemical activity. It is then transplanted to a new culture medium. In actively growing tissues this may have to be done every second to fourth day. The medium consists of Tyrode's solution with extract of beef embryo as a stimulating substance, and serum and plasma derived from the animal in which the graft is to be placed. Enough serum and plasma to form a coagulum of fibrin are always used.

Toxic goiter; an analysis of the results of surgical treatment. Wallace, H. L. and L. B. Wevill, Edinburgh M. J. **40**: 598. 1933.

This is a review and careful analysis of 285 cases covering a period of ten years and checked as to present condition in 251. The cases are grouped into primary toxic goiter and secondary toxic goiter. Between 75% and 80% of the patients are fit for work and almost symptom-free. The mortality rate for all types of cases was 12.3%. No special mention is made of iodine therapy.
—J. C. D.

Hodgkin's disease simulating adenoma of the thyroid gland. Warnshuis, Lillian, M. Clin. North America, **17**: 297. 1933.

The patient described was a white female of 15 who was admitted to the hospital in 1931. The chief complaints were nervousness of two years' duration, emotionalism, swelling of the front of the neck, and difficulty in swallowing of two weeks' duration. Other symptoms were insomnia and night sweats for the past two or three months. There were no outward signs of nervousness or exophthalmos; there was no loss in weight; the temperature was normal, and the pulse rate was 110. In the suprasternal notch there was a swelling the size of an egg, apparently originating in the thyroid, giving rise to a diagnosis of adenomatous goiter. The basal metabolic rate was minus 7%. There were no other significant signs, symptoms or laboratory evidence that would refute the diagnosis of thyroid adenoma, hence thyroidectomy was decided upon but postponed until the second admission to the hospital four months later. At that time there were such pressure symptoms as choking sensations, a hacking cough, and paroxysmal attacks of dyspnea. The swelling was larger and had apparently extended substernally. A partial thyroidectomy was performed. The gland tissue was discovered to be replaced by an avascular, irregular, cartilaginous mass. The pathologist's diagnosis was Hodgkin's disease. A third admission to the hospital because of a return in aggravated form of former symptoms a few months later revealed the patient to be acutely ill with typical Hodgkin's disease. The patient's condition was somewhat ameliorated

by treatment, especially radiation. A fourth admission two months after the third proved the patient to be in moribund condition, and she died 37 days later. Hodgkin's disease of the thyroid gland is rare. Prior to operation the x-ray examination did not show any substernal masses. On the third admission there was evidence of a mass in the mediastinum, and deep x-ray therapy proved futile.—I. B.

Mild hypothyroidism. Watkins, R. M., Ann. Int. Med. **7**: 1534. 1934.

Fifty patients, mostly females, have been studied for the purpose of demonstrating some of the salient points of mild hypothyroidism. The name of the disease may be incorrect; rather, the condition may be a syndrome in which abnormalities of other organs, besides the thyroid gland, play a part. The condition is frequently present in people of middle age. Evidences of the disease are vague in that there are no specific symptoms. Outstanding in these complaints are the indefinite loss of energy, nervousness, mental apathy, extreme fatigue, loss of muscle strength, general weakness, increase of weight, constipation and so on, of which so many women complain. The basal metabolic rate is lowered to a point moderately below normal. There are no characteristic findings on physical examination. Those most frequently discovered are a moderate degree of overweight, rapid pulse, normal blood pressure, unusual dryness of the skin and hair, and frequently various associated diseases usually of chronic type. It is of some interest to note that a majority of these patients had had an abnormal number of potentially serious ailments in their earlier years. The average number of children born to the married women of the group was well below that of the race in general. A strikingly large proportion of these patients had been operated upon for various conditions. It is possible that many of the operations were carried out in an effort to relieve these people of their vague complaints. It is concluded that mild hypothyroidism is a disease which is unquestionably common. It often remains undiagnosed or is improperly diagnosed for a long period of time. The disease has no clear-cut, specific symptoms or signs.—From Author's Abst.

PRINTED IN U. S. A.